CASE REPORT

SPECIES: Humpback Whale (*Megaptera novaeangliae*)

DATE: FIRST OBSERVED: April 6 2010

DATE EXAMINED: April 9 2010

CASE NUMBER: NY4236-10

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***Executive Summary.***

On the morning of April 6, 2010, the local marine mammal stranding network organization reported a live 30 foot 13 ton juvenile humpback whale (*Megaptera novaeangliae*) beached in heavy surf in East Hampton, NY. Humpback whales are protected under both the Marine Mammal Protection Act (MMPA) and the Endangered Species Act (ESA) since they are listed as "endangered" under the ESA. A team of marine mammal stranding response and veterinary experts responded under the direction of NOAA and in consultation with a larger team of experts nationally. The team assessed the situation and determined that the size and mass of the whale, coupled with the environmental conditions at the time, prevented any type of intervention such as pushing or pulling the animal out to sea. After evaluating all the options the team determined the most humane and best course of action was to euthanize the whale. Over the course of several days, the team made arrangements to carry out humane euthanasia and a subsequent post mortem examination and carcass disposal. Based upon evaluation of all the facts such as the gross observations, histological evaluation of tissues, ancillary diagnostics, and environmental issues, no definitive cause of the stranding was determined.

***Background.***

Individual and mass strandings of cetaceans are common and have been attributed to such causes as infectious disease, biotoxin exposure, human caused activity (e.g. ship strike or fishing gear entanglement), or age-related mortality (e.g. "natural causes"). Often, however, the cause of stranding is not determined due to many factors including decomposition state of the animal carcass, logistical constraints for evaluations, or there is no apparent evidence of significant disease or injury upon examination. This whale was severely compromised by the stranding event so the team decided to humanely euthanize the whale to end its pain and suffering. The size and mass of the whale, coupled with the environmental conditions at the time and the failure of the animal to refloat during successive tidal cycles prevented any other type of intervention. Details about the event and case investigation are given in this report.

The NOAA's National Marine Fisheries Service (NMFS) Marine Mammal Health and Stranding Response Program (MMHSRP) was created in 1992 through a statutory amendments to the MMPA. Through this, the NMFS MMHSRP is designated as the lead to coordinate response to stranded whales, dolphins, seals and sea lions and to investigate the causes of strandings. The MMHSRP partners with stranding networks throughout the United States to monitor for, respond to, and investigate stranding events. The MMHSRP holds a scientific research and enhancement permit under the MMPA and the ESA for stranding response and investigation (including sample and tissue collection) of endangered marine mammals, including large whale species (Permit No. 932-1905-MA-009526). More information on the MMHSRP can be found at: http://www.nmfs.noaa.gov/pr/health/.

As part of a stranding investigation, a standard set of information is collected including circumstances of the stranding event (e.g., location of stranding, environmental conditions, human activity, etc.), external and internal exam. The exam typically

includes a necropsy which is conducted by trained experts of the stranding network and includes an assessment of organs and body condition (gross necropsy) and collection of samples for microscopic evaluation (histopathology), life history information (morphometrics, age, genetics, contaminants), and ancillary diagnostics such as microbiology, virology, and toxicology (biotoxins, chemicals or anthropogenic pollutants).

***Case Overview for Humpback Whale on Long Island (ID No. NY4236-10)***

On the morning of April 6, 2010, the local NMFS authorized marine mammal stranding network organization reported a live humpback whale (*Megaptera novaeangliae*) beached in heavy surf in East Hampton, NY. Humpback whales are protected under both the MMPA and the ESA. Response efforts for this stranded humpback whale were conducted under Permit No. 932-1905-MA-009526 issued to NOAA. The size and mass of the whale, as well as the challenging environmental conditions on Long Island, prohibited rescue efforts and required that the whale be humanely euthanized to end its pain and suffering on the beach. An expert Response Team led by NMFS MMHSRP was assembled to evaluate the whale and to set a course of action. The Response Team evaluated several options including tide release, towing out to sea for release, humane euthanasia or natural death. In consultation with marine mammal veterinarians and biologists nationwide, the Team decided the only logistically feasible and humane option was euthanasia. In consultation with expert marine mammal veterinarians, biologists, and a veterinary pharmacologist, the Team administered a combination of pharmaceuticals and ballistics to sedate and subsequently euthanize the whale.

*Euthanasia.* The following single or combination of drugs and methods of administration were utilized for euthanasia:
1.Midazolam at 0.2mg/kg IM/Butorphanol at 0.2mg/kig IM. (7th April 2010).
2.Butorphanol 6000 mg IM. (8th of April 2010).
3.Beuthanasia-D 600ml IP and 320 ml IV (retrobulbar plexus) after 3 pericranial .577 ballistic rounds. (9th April 2010).

On April 9, 2010 at 11:45AM, the whale expired and an expert team of experienced large whale biologists and veterinary pathologists conducted a necropsy over a 5.5 hour period. Numerous samples were collected for scientific analysis and the carcass remains were transported from the beach site by the Town of East Hampton Sanitation Department and the beach was cleaned by the necropsy team as directed by Long Island officials.

***External Exam.***

The humpback whale was a juvenile (1-2 year old) male that was over 30 feet long and weighed around 13 tons (more than 26,000 pounds), which is the approximate size and weight of a large passenger city bus. It was in good body condition based upon the subcutaneous and visceral adipose and musculature. The sites of pharmaceutical injection by dart were evident and extended into the blubber and muscle. In addition, the site of ballistic entry on the right side was present.

***Internal Exam***.
A large accumulation of adipose was present within the thoracic cavity which covered the heart and partially compressed the right and left lung lobes. Fluid but little solid material was present in the intestine. Within and around (retroperitoneal) the kidneys, there were round worms with associated tissue reaction (i.e., granuloma). Near the site of the ballistic entry, the right ear was fractured and in the underlying brain, there was focal, subdural hemorrhage. There was discoloration, focally, of the abdominal muscle.

Samples from all organs were collected and submitted for microscopic evaluation. Samples were also collected for virology, microbiology, chemical, and biotoxin analysis. The brain was collected *in toto* by Mount Sinai for radiographic study and subsampling of the brain was conducted for microscopic evaluation.

*Microscopic Findings*
A total of 89 tissue samples and whole brain were submitted and examined by two board-certified veterinary pathologists, Drs. Dee McAloose (Wildlife Conservation Society) and David Rotstein (NOAA). The pathologists examined the same set of tissues through the generation of duplicate slides.
Microscopic findings of note include confirmation of the presence of granulomas in the kidney and retroperitoneal space. In addition, there was inflammation of the abdominal fat (mesentery), periureteral fat, and peripancreatic fat. In the liver, there were lipid (fat) vacuoles and glycogen (storage form of glucose) within hepatocytes. In the bronchioles of the lung, there were inflammatory cells, increased mucus, and occasional bacteria. There was focally extensive subdural hemorrhage in the occipital lobe thought to be secondary to ballistic impacts nearby. There was hemorrhage within the underlying cerebrum and perivascular hemorrhage throughout the brain. In the abdominal musculature, there was degeneration of collagen and myofibers. The right prescapular lymph node contained abundant blood.

***Ancillary Diagnostics and Anthropogenic Evaluation***

*Environmental Data*
Weather and tide patterns were normal.

*Military Activity*
NMFS MMHSRP requested information from the U.S. Navy about military activity in the vicinity around the time of the stranding. The Navy reported no active sonar within 100 nm and previous 72 hours. There were no reported ship strikes. The closest reported activity was a single unit approximately 197NM from the location of the stranding on the evening of April 4, 2010. The next closest was 3 units conducting Unit Level Training ~220NM of the stranding at various times from between March 31, 2010 to April 5, 2010.

*Radiography*

A computerized tomography scan (CT) was performed at Mount Sinai to evaluate the brain as part of an ongoing research project. The site of subdural hemorrhage observed at necropsy was present and extended into the associated cerebral sulcus.

*Virology*
Routine evaluation of morbillivirus infection was performed utilizing a"Universal" morbillivirus PCR at the Athens Veterinary Diagnostic Laboratory on July 7, 2010 and results showed no evidence of the virus in submitted tissues (Accession Number A110641).

*Microbiology*
Bacterial culture of select tissues was conducted by a private veterinary diagnostic laboratory, Antech Diagnostics. There were no microbiological isolates of concern found in this animal.

*Urinalysis*
An urinalysis which included evaluation of protein, glucose, specific gravity, blood, ketones and urine myoglobin was performed at the Texas Veterinary Medical Diagnostic Laboratory The results were within normal limits for a cetacean and no urine myoglobin was found.

*Complete Blood Count and Serum Chemistry Analysis*
A complete blood count (CBC) and white blood cell count (WBC) were completed at a private veterinary diagnostic laboratory, Antech Diagnostics. Reference ranges for humpback whales have not been developed therefore evaluation of this case required comparison to values of other marine mammals, specifically small cetaceans. For this reason, an "increased" or "decreased" value should be considered based on an inter species comparison, be regarded with caution, and be qualitative at this time.

Based on comparisons with dolphin reference ranges, there were mild decreases in the following: red blood cell count, hematocrit, white blood cell count, serum phosphorus, and potassium. There was a mild increase in serum calcium. All other values were within the reference ranges of other cetaceans.

*Steroids and Vitamins*
Plasma testosterone was <40.0 pg/ml and Vitamin A was 50.0 ng/ml.

*Parasitology*
Renal parasites collected in necropsy were fixed in 100% ethanol. The parasites were submitted to Dr. Williams at the National Marine Life Center and to the Cornell University Animal Health Diagnostic Center. Both identified the parasites as a nematode, *Crassicauda boopsis*, a parasite frequently observed in the kidney and associated vasculature of large whales.

*Biotoxins*

Analyses for biotoxins (okadaic acid, saxitoxin, brevetoxin, and domoic acid) were conducted at the National Ocean Service, National Oceanic and Atmospheric Administration, Marine Biotoxin Program. Samples of bile, urine, stomach contents, and intestinal contents were negative for any of these biotoxins.

***Summary of Findings***

The immediate cause of death of this stranded cetacean was euthanasia resulting in cardiopulmonary collapse. Based upon gross observations, histological evaluation of tissues, environmental conditions, and ancillary diagnostics, a cause of stranding was not determined in this case. There was no evidence of infectious or inflammatory disease or biotoxicosis. Individual and mass strandings of cetaceans are not uncommon in the coastal US. While, in some cases, strandings may be attributed to such causes as infectious processes, biotoxin exposure, anthropogenic activity, or age-related mortality (e.g. “natural causes”), in many cases, the cause of stranding cannot be explained as there is no evidence of significant disease or environmental changes.

There were findings of note in this case including the grossly observed mass of adipose in the thoracic cavity, renal endoparasitism, acute inflammation in the airways, focal subdural and parenchymal hemorrhage in the brain (likely a result of concussive forces from the ballistics), hepatic lipid and glycogen accumulation, and myofiber and collagen degeneration in the ventral abdominal musculature. These findings may be separated into chronic processes and acute-likely post-stranding related processes.

The fat body in the thoracic cavity was composed of mature adipocytes with no evidence of inflammation or necrosis of fat. Given that this animal was in good body condition, this fat body may be a normal anatomical feature. That said, in a beached stranded animal of this size and weight which places great pressure on the thoracic cavity, this fat body could affect cardiopulmonary function to some degree.

Glycogen, a storage form of glucose, was increased in the liver. This increased glycogen could reflect endogenous production of cortisol resulting in a “steroid hepatopathy”. This is a likely indicator of a stress response. Lipid was also present in hepatocytes which could reflect mobilization of fat stores in an animal that has not been eating. Gastric contents were minimal and while the animal did not eat while on the beach, it is not known whether feeding was inhibited immediately prior to stranding, but the good body condition suggests it had been feeding in recent weeks or months at least.

The focal subdural hemorrhage with extension into the superficial cerebrum as well as the fractured ear likely relate to the ballistic trauma, though other cases of an animal rolling the surface has shown head trauma including fractures. It is unlikely that the hemorrhages occurred prior to or early in the stranding as the cellular constituents of the hemorrhage was erythrocytes without evidence of clot formation or macrophages infiltrating the site of hemorrhage. This indicates that the hemorrhages occurred shortly before death or around the time of death.

The inflammation in the large airways in the lung likely occurred during the course of stranding reflecting decreased respiratory clearance in a compromised animal. Degeneration of muscle fibers and collagen are likely the result of pressure on the abdomen leading to localized damage.

In summary, the cause of the stranding was not determined based on histological examination of tissues, diagnostic testing, and environmental assessment.

# APPENDIX 1 - GROSS REPORT

Field # NY4236-2010 *Mn*

## GROSS NECROPSY REPORT

**SPECIES** *Megaptera novaeagliae* **FIELD NUMBER** NY4236-2010

**LOCATION** East Hampton, NY **LAT** 40.9454N **/LONG** -072.1870W

**DATE** 6 April 2010 **GENDER** M **TOTAL LENGTH** 939.8cm

**HISTORY:**

**Summary of the East Hampton Humpback**
***Tuesday April 6th 2010***. A live humpback beached in East Hampton NY in heavy surf.

***Wednesday April 7th***. Given the surf conditions and the desire to avoid barbiturate residues, over-sedation was considered as a first step to avoid or at least minimize the need for barbiturates, using the delivery system recently developed for entangled large whales. The plan, developed with Mike Walsh was to administer 2x the sedation dose developed for disentangling right whales: O.1 mg/kg of both butorphanol and midazolam. Respective reversants – naloxone and flumazenil were on hand in case of accidental human exposure. That evening EMT's on the beach were unauthorized to use flumazenil in the event of accidental human exposure, so the decision was made to only use butorphanol. Whale continued to be grounded in high surf all day.

***Thursday April 8th***. At 0015 this situation was communicated to Michael Moore, and he recommended to use the entire available stock of butorphanol: 120ml of 50mg/ml. In light of the 30' total length measured at necropsy which puts the weight at around 13,000kg, this was a dose 4x that used in the recent right whale entanglement case. The effect was to increase the respiratory rate, but otherwise the animal remained bright and alert. That same evening Paul Calle (WCS) worked with David Morin, and Riverhead staff to deliver a mixture of butorphanol, medetomidine and midazolam with a resulting rapid and profound sedation and hypoventilation. This was followed by more meperidine and midazolam an hour later. Then a loaded dart bounced off and was lost in the surf, at which point further darting ceased, given the concern of dart loss recurring. Riverhead remained at the site to monitor the animal. Earlier in the day a meeting in WV had discussed the option of involving Allen Ingling, an experienced wildlife marksman/ veterinarian with substantial experience of using rifles with baleen whales, of using a .577 rifle, to avoid generating a barbiturate residue, and to enable euthanasia with the surf still running. The meeting also discussed the need to assemble a necropsy team to gather samples for drug residue analysis and undertake a thorough necropsy. As a result, Trevor Spradlin, Jamison Smith, Bill McLellan, and Alex Costidis departed by road at 16:00, and Michael Moore, Allen Ingling and Steve Raverty by road at 1700.

***Friday April 9th*.** 0000-01:00 No respirations were observed over the duration of an hour. However at 0755 the animal was moving its eyelids and weak respirations were again apparent. There was no palpebral reflex and the animal was motionless other than weak respirations. It was essentially anesthetized at this point. Discussions on the beach and

with NOAA resulted in agreement to use the .577 rifle. Three rounds were dispatched, using the New Zealand DOC guidelines for landmarks to target the brainstem. At 0900,with the surf subsided, 600 ml of euthanasia solution were then administered using a 6" needle in the ventral abdomen, and a further 320 ml with a 4" needle, in the retrobulbar plexus. The last respiration was noted at 11:45.

Moore spent the morning working with Jamison Smith and Trevor Spradlin to undertake the necessary public information dissemination about the lost dart. This began as a meeting with the local police department and the mayor of East Hampton. An empty dart and needle was passed around. We suggested that the pressure retaining sleeve had likely shifted on impact and the drug discharged, and thus the chances of a loaded dart being a risk to humans was very low, but nonetheless significant. The dart contained medetomidine, meperidine, and midazolam. Our willingness to communicate our concerns frankly was much appreciated. We were asked to do the same with the Suffolk County Health Department. This was done via conference call thereafter, with Teri Rowles and David Brunson also on the phone. As a result of this a written summary of the drugs involved was provided to the health department along with a photograph of the dart. The county then passed this information on to regional EMT services. Flyers were prepared for distribution. A search for the dart was undertaken with no success to date. At 1600 a press conference was undertaken by NOAA, Rob DiGiovanni, Michael Moore and the local police department.

A necropsy was undertaken that afternoon, by Riverhead, Bill McLellan, Stephen Raverty, Alex Costidis, Denise McAloose, Alisa Newton and a crew from WCS. The necropsy started at 13:00 and was finalized at 18:30 and the beach clear, with the remains of the whale removed by the town of East Hampton in a dumpster. All field gear was brought back to Riverhead for cleaning and the beach was dragged flat by 19:00.

NY 4326-2010 *Mn* immediately after drawing up from surf

NY4236-2010 *Mn* Left side

NY 4236-201 *Mn* head-on view

**GROSS EXAM:**

A 1-2 year old male humpback whale (*Megaptera novaeangliae*) is presented euthanized, April 9, 2010 in good post mortem and body condition. Numerous large (up to ~5cm diameter) barnacles are present rostral tip of the gular pleats and along the cranial margins of the flippers. Numerous cyamids are present on the skin of the cranial half of the animal. There are ample subcutaneous and abundant visceral adipose stores and the animal is well muscled. Considerable adipose is seen investing the epaxial muscle. Throughout the carcass, there is generalized pallor of the tissues. Within the right paralumbar region, there are 3 clusters of dart injection sites. The first and most ventral consists of 3 sites, the second is more dorsal, midlateral and features 6 multifocal to confluent penetrating sites and the third and most dorsal cluster has 4 injection sites.

NY4236-2010 *Mn* Sites of darting and tissues collection

Within the third injection site region, the three cranial injection tracks are diagonal and 5.0-5.2 cm long with the most dorsal tract, vertically deviated and extending to the distal limit of the dorsal fin as a superficial cutaneous furrow (deflected and lost dart). Within the hypodermis and extending a short distance into the underlying skeletal muscle of the second cluster of injection sites, there is focally extensive acute hemorrhage and edema fluid which tracks dependently a short distance ventral to the injection sites. The cluster of injection tracts of the third (most dorsal) injection site extend to the mid to ventral third level of the blubber. Immediately caudal and extending approximately 0.5 m from the rectum, there is marked, subcutaneous hemorrhage and clot formation within the midventral region of the peduncle. Approximately 0.5 and 1.0 m cranial to the anus, there are individual transverse linear cutaneous constriction bands overlaid by intact skin (possible human interactions).

**INTERNAL EXAM:**

Blubber thickness

| | |
|---|---|
| Dorsal at nuchal crest | 8.1 cm |
| Mid-lateral at nuchal crest | 9.3 cm |
| Mid-lateral at mid-thorax | 7.3 cm |

Within the right lateral aspect of the neck, along the level of the cranial insertion of the right flipper and lateral canthus of the eye, there are 2 circular and 1 elliptical gunshot entrance wounds with associated subcutaneous hemorrhage and cavitation. The most ventral and cranial entrance site is designated number 1; the defect is irregular and horizontally elliptically. The second entrance site is circular, 20 cm caudal to the first, but is slightly more dorsal. The most dorsal entrance wound, designated number 3, is 130 cm from dorsal midline and the trajectory is diagonal, dorsal to the vertebral column and the bullet was lodged in the deep blubber, contralateral to the entry wound.

NY4326-2010 *Mn* ballistics sites

NY4236-2010 *Mn* ballistics morphometrics

Extending along the entire length of the right mandible medially and throughout the ventrolateral aspect of the synchondrosis, there is marked focally extensive subcutaneous hemorrhage, which multifocally dissects along fascial planes and extends a short distance into the adjoining skeletal musculature of the tongue and intrinsic laryngeal musculature. Approximately 60-80 cm distal to the left flipper insertion, along the midventral aspect of the appendage, there is a large 25-30 cm x15-20 cm longitudinal ulcer with abrupt, serpiginous margins and the exposed dermis is moderately congested; within the centre of the defect, there is a lacerated small calibre artery which oozes blood. Immediately caudal and parallel to this defect, there is a smaller, superficial erosion with curvilinear and more graded margins. At the right lateral aspect of the caudal abdominal wall, there is a large, 0.8-1.0 m wide white parabolic band and along the leading edges of the right and left caudal flukes, there were numerous transverse and oblique white striations. A small number of copepod parasites are localized deep within throat pleats and vibrissae are evident.

**Respiratory system:** Throughout the entire length of the ventral half to third of the thoracic cavity, enveloping the pericardium and dorsocaudally elevating and moderately compressing both lung lobes, there is massive accumulation of adipose tissue. The superficial aspect of this fat featured a prominent undulating to complex mosaic pattern and on sectioned surface the tissue is homogeneous pale grey white. Approximately 10 cm proximal to the distal limit of one fat lobule, presumably corresponding to the portion of fat protruding through the thoracic inlet, there is a 2 cm x3 cm well delineated elliptical hemorrhagic defect with cavitation of the adjoining stroma (presumptive bullet tract). There is approximately 20 ml of pale red serous fluid within the thoracic cavity with numerous refractile surface lipid droplets. The lungs are diffusely soft, pliable, red and small amounts of blood drain from cut surfaces. All sectioned tissue floats in formalin. Extending from the tracheal bifurcation caudally deep into the parenchyma, there is a moderate accumulation of stable white froth. Approximately 1L of thick white froth expresses from the larynx into the pharynx upon manipulation and removal of the lungs. The laryngeal cartilages appear unremarkable.

**Hemolymphatic tissues:** Multiple lymph nodes from the mediastinal, retropharyngeal and abdominal regions are moderately to markedly enlarged and on cut surface, are glistening and homogeneous pale brown to dark red brown with recognizable cortical and medullary tissues. Within the retropharyngeal region, there are a small number of lymph nodes which on cut surface feature subcapsular and medullary (draining) hemorrhage. Mesenteric lymph nodes all contain prominent, discrete, white-tan, foci that bulge on cut section and vary in size from 6 to 12 mm diameter within the cortex (lymphoid follicular hyperplasia, presumptive). Within the mesenteric adipose tissue, approximately 3 cm from the right ventral limit of the spleen, there are two discrete, ovoid, nodules of tissue that on cut section have a similar consistency to the spleen. These foci are approximately 3 x 4 x 2 cm and 2 x 3 x 2 cm. The larger focus has a central depression with a region of thickening of the capsule.

**Digestive system:** There is moderate distension of the stomach, which is fluctuant on ballottement and contains approximately 50 L of pale green clear fluid with a small

amount of dispersed flocculent white material (mucus or exfoliated mucosa). Along the entire length of the right lingual groove within the oral cavity and interspersed within rugae of the first stomach compartment, there is a moderate amount of sand. There are two bilaterally symmetrical regions of trabecular tissue on the luminal side of the laryngopharynx, along the lateral aspect of the larynx, similar to the lymphoid aggregation in the pyriform fossa of odontocetes. The left structure appears eroded and discoloured. Two narrow, elongate linear erosions, one on each of the dorsal margins of the epiglottic folds are present. A small, approximately 0.5 cm diameter, slightly raised, white, vesicular structure that contains a small amount of clear, serous fluid is present in the mucosa just rostral to the left epiglottic fold. Approximately 3 cm distal to the junction of the 1st and 2nd chambers of the stomach is a small, light tan, firm, 0.3 cm diameter, nodular swelling of the mucosa. Involving approximately 1 m of bowel within the mid-region of jejunum, along the antimesenteric border, there is multifocal to coalescing subserosal hemorrhage and throughout the length of the small and large intestine, there is a moderate amount of bright tan brown to green yellow fluid ingesta and the mucosa is diffusely tan brown. The liver measures approximately 138.5 cm x 87 cm x 24 cm. The parenchyma is diffusely dark red to brown on cut section.

**Urogenital system:** This is a male whale. The right testis is 13 cm long x 12 cm (circumference). The tissue is soft, light tan and bulges slightly on cut section. The left testis is not found. The mucosa of the penis and prepuce is smooth and light pink/tan. The right kidney is 91 cm x 38cm x 11cm and weighs 20.5 kg; the left is 87 cm x 37 cm x 11 cm and weighs 21.8 kg. Deep within the parenchyma of both kidneys within several ureters are multiple (5 – 10), 0.1 cm diameter, red, round nematode parasites. Along the craniomedial aspect of the right kidney, loosely adherent to the peritoneum, there is a 2 cm encapsulated abscess. The urinary bladder is markedly distended with clear pale yellow urine.

**Nervous system:** There is a closed comminuted fracture of the right ear with minimal to mild displacement of bone fragments and scattered periosteal hemorrhage (gunshot?).

NY4236-2010 *Mn* Right bulla fracture

The fracture involves the attachment of the bulla to the earbone complex. Small bone fragments and blood and chicken fat clots are present within the middle ear cavity. Tissue damage is not observed in the tissues immediately surrounding the earbone complex or in the highly vascularized fibrovenous plexus associated with the pterygoid and peribullar sinus, thought there was a region of the right occipital condyle, on the right lateral opening of the foramen magnum, of dark red clotted blood. The clot projected approximately 3cm deep into the condyle with clear margins and was not apparent in the left condyle. The right "glove finger" is exposed and collected in tact for aging. Involving the caudal third of the right cerebral hemisphere (occipital), There is focally extensive subdural hemorrhage with poorly delineated margins and multifocal epidural fibrin deposition.

NY4236-2010 *Mn* Brain hemorrhage

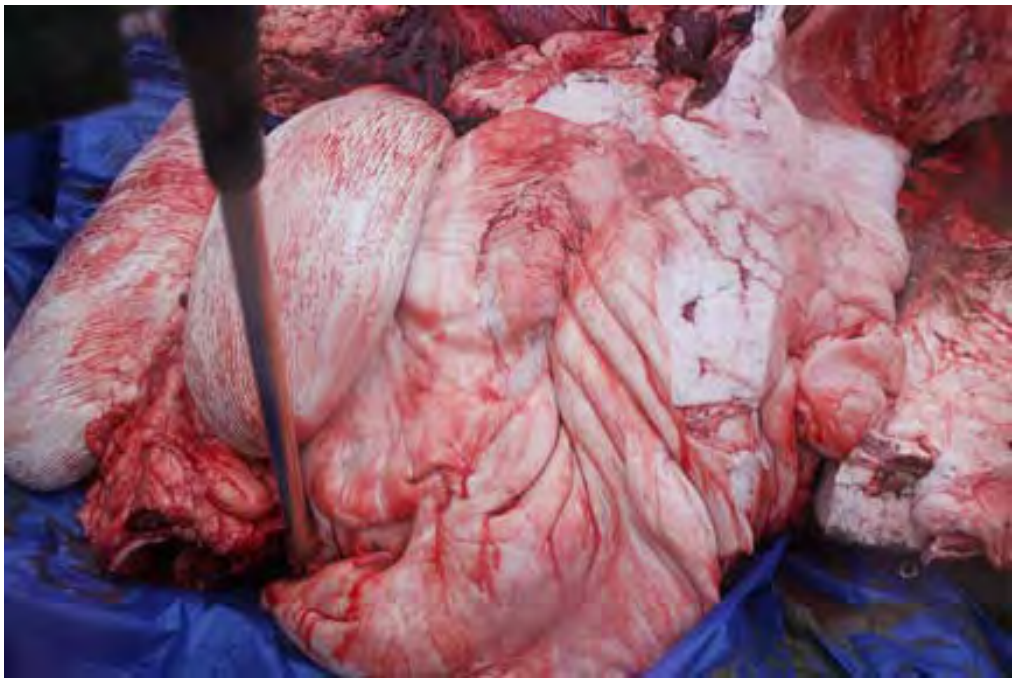
Throughout the superficial aspect of the contralateral hemisphere, the meningeal vasculature features multifocal to coalescing clear (gas) intravascular bubbles with scattered perivascular edema and hemorrhage. The retrobulbar extra-ocular tissue of the right eye is diffusely edematous and features scattered foci of acute hemorrhage (injection site of euthanasia solution?).

**Musculoskeletal system:** Within the right ventral aspect of the mid-abdomen, there is focal necrosis of the hypodermis with erosion of the apposed internal aspect of the blubber, attached fibrous strands, and extensive edema with scattered hemorrhage within the adjoining abdominal rectus musculature.

NY4236-2010 Mn multifocal myocellular degeneration and necrosis with occasional fibrin deposition

On sectioned surface the internal and external rectus muscle, there is multifocal myocellular degeneration and necrosis with occasional fibrin deposition, acute hemorrhage and variable accumulation of edema fluid. Similar, but less extensive changes are apparent within the lumbosacral epaxial skeletal musculature.

**Cardiovascular:** The heart is surrounded by abundant adipose tissue in a mass approximately double of both lungs and 4x times that of the heart.



NY4236-2010 *Mn* Thoracic mass 2x times the size of both lungs

There is no blood in the aorta; minimal clotted blood is present in the chambers of the heart. There is a thickened fatty tissue engulfing the entire left ventricle.

NY4236-2010 *Mn* Heart

The ductus arteriosus is tightly contracted down but is probe patent. Contracted (scar) tissue with peripheral red streaking is present at the site of the closed foramen ovale. The right and left atrioventricular valves are 79 cm and 58.5 cm in circumference, respectively. The pulmonic valve is 47 cm in circumference and the aortic valve is 40 cm in circumference. The thickness of the right ventricular free wall is 3.5 cm and the interventricular septum is 7.5 cm; that of the left ventricular free wall at the level of the moderator band ranges from 7.5 cm – 9.5 cm within a 4cm length at the level of the moderator band.

NY4236-2010 *Mn* Heart wall thickness

**Endocrine:** There are no overt lesions within the endocrine system. The right adrenal gland had normal cortico-medullary distinction. The left adrenal gland was not found. The pituitary gland is light tan with slightly darker tan edges.

**Eyes:** There are no overt lesions in the L eye (the R eye was collected whole).

**GROSS FINDINGS:**

1). Carcass: Obesity, marked, generalized
2). Carcass: Possible anemia, generalized (hypovolemic shock)
3). Thoracic cavity, ventral third to half: Fatty deposition, severe, diffuse with caudodorsal displacement and compression of the lung lobes
4). Lymph nodes, multisystemic: Lymphadenopathy/lymphadenomegaly, moderate, multifocal with occasional extracapsular edema fluid and draining hemorrhage
5). Ear, right: Fracture, comminuted, closed, with minimal displacement of bone elements and peri-otic hemorrhage (bullet tract)
6). Brain, right caudal cerebral hemisphere: Hemorrhage, subdural, moderate, focally extensive, acute
7). Brain, meninges, left cerebral hemisphere: Gas bubbles, intravascular, moderate, multifocal to coalescing with scattered perivascular hemorrhage and edema fluid (possible embolization)
8). Fascia, left mandibular body and isthmus: Hemorrhage, subcutaneous and fascial, marked, focally extensive, acute
9). Abdominal wall, right ventral: Cellulitis, moderate, focal, fibrinonecrotizing, with multifocal myocellular degeneration and necrosis of the adjoining abdominal rectus musculature and necrosis of the overlying blubber
10). Skin, midventral abdomen: Constriction bands, moderate, bifocal, linear (possible human interaction)
11). Kidney: Nematodiasis, encapsulated, mild, focal, subacute
12). Subcutis, proximal, midventral peduncle: Hematoma, longitudinal, moderate, focal, acute
13). Stomach, nonglandular compartment: Pica, moderate, focal (ingested sand)
14. Ductus arteriosus: Contracted but probe patent.

**COMMENTS:**

This animal presented with a history of live stranding Tuesday, April 6, 2010 and was euthanized by a combination of sedation administered Thursday, April 8, 2010 at approximately 10:30 pm, 3 gunshots to the right lateral neck region at approximately 8:30 am, and ultimately intramuscular and retrobulbar barbiturate administration Friday, April 9, 2010 at approximately 9:00 am. The myocellular degeneration and necrosis of the abdominal and epaxial skeletal musculature, focal cellulitis within the right ventral abdomen, subcutaneous hemorrhage along the ventral aspect of the peduncle and ingested salt water are likely sequelae to beach casting and substrate impact associated with rolling within the surf rather than contributing factors in the stranding event. Cumulatively, these changes would have contributed at least moderately to antemortem morbidity. Based on the generalized pallor of the viscera, hematocrit of the agonal blood sample, and lack of blood within the aorta, hypovolemic shock is suspected. The

shattered right ear and subdural hemorrhage are suspect trauma from gunshot, though blunt trauma from rolling in the surf could have been the cause as well. Histopathology of the right cerebral hemisphere may provide additional insights into the timing of neural injury. Throughout the dorsolateral aspect of the cervical region, the bullet path is associated with crushed and occasionally hemorrhagic tissue, which may be related to tumbling, yawing and deformation of the bullet (permanent cavity), as well as the sonic shock wave of the bullet, resulting in expansion and injury of the tissue cavity (temporary cavity). The gas emboli throughout the meninges of the left cerebral hemisphere is unusual and in this case may be artefact, related to introduction of air into the tissues due to cavitation associated with the bullet entrance and transit through the tissues, trauma to the trachea or larynx with secondary introduction of air into major blood vessels, or some other mechanism; gas bubbles were not seen grossly in other sites. In other deep diving cetaceans, the accumulation of intravascular gas bubble formation has been attributed to rapid ascent from depth. Due to time constraints to complete the necropsy and size of the animal, radiography could not have been undertaken and localization of the remaining bullet was unsuccessful. Throughout the entire length of the thoracic cavity, there was massive accumulation of adipose tissue with dorsal displacement and compression of both lung lobes. This finding is unprecedented based on the experience of the necropsy team and lead prossector (WAM). The physical size may have impacted normal cardiopulmonary function, contributing to the live stranding of this animal. Further evaluation awaits histopathology and ancillary studies. The number of renal parasites was small and presumably would not have contributed to impaired renal function; specimens have been collected and will be forwarded for speciation. The pica may be inadvertent, associated rolling within the surf, generalized debilitation associated with metabolic exhaustion, lack of available feed or some other process. Within the caudal limit of the oral cavity, there were scattered superficial mucosal vesicles. Although incidental in this case, the lesions are suggestive of a possible viral infection and follow up molecular studies will be pursued to identify a specific etiology. The generalized lymphadenopathy is suggestive of low to intermediate grade antigenemia, possibly associated with or exacerbated by generalized debilitation or immunosuppression due to prolonged time in the surf. The intestinal hemorrhage is anatomically associated with the ventral abdominal wall cellulitis and may reflect a localized traumatic event, infection, inflammation, partial strangulation, or some other process.

The morphometrics data and collected tissue samples are appended to this report. There were a total of 2388 images taken of this live stranding and necropsy operations over the four days of this investigation. The vast majority of these images are not presented in this gross report but are available from Riverhead Foundation for Marine Research and Preservation or the Necropsy Team leader. The assistance of the Riverhead staff and volunteers during the necropsy is gratefully acknowledged by the necropsy team.

**CURRENT RESULTS**

**URINALYSIS (provided by WCS)**
**Strip:**
Glucose: Negative
Bilirubin: Negative
Ketone: Negative
S.G. (refractometer): 1.023
Blood: Negative
pH: 6
Protein: Negative
Urobilinogen: Negative
Nitrate: Negative
Leukocytes: Negative
Description: pale yellow, slightly turbid

**Direct microscopic (Sedi-stain)**
WBC: Negative
RBC: Negative
Epithelial cells: Occasional
Casts: Negative
Crystals: Negative
Other: Moderate amount of amorphous debris

**PENDING:**
Histopathology, Dr Rotstein and Dr McAloose
Aerobic culture
Post mortem heart blood for Brucella serology
Trace mineral and vitamin analysis, liver
Gross photos recorded
Right eye and extra-ocular adipose tissue of left eye recovered for barbiturate
Three dart cluster sites excised separately with 3-5 cm border, placed en masse in foil, and frozen for chemical analysis
6-8 cm rectangular full thickness skin and blubber blocks collected 0.5 and 1.0 m cranial, caudal and ventral to the margins of injection sites for chemical analysis
80 ml of serum collected and archived in 1 ml aliquots, 3 ml of whole blood and 2 ml of whole blood of potassium EDTA
Blowhole and exhaled air swabs submitted for culture and PCR
Lung, spleen and thymus, PCR for morbillivirus and Brucella
Blubber, liver and kidney for contaminant levels
Multiple tissues retained at -80C
Fecal floatation
Stomach contents retained
Bile collected
Vibrissae retained
Vesicles, fluid and membranes retained

One bullet recovered
Brain, preserved in para-formaldehyde for Dr J Reidenberg, Mount Sinai, NY
Right ear, preserved in formalin for Dr T Cranford
Cyamids retained
Cerebrospinal fluid collected
“Glove finger” for aging
Multiple baleen plates collected at Mount Sinai

**This report was generated by:**
Dr. Stephen Raverty
Dr. D. McAloose
Dr. Alisa Newton
Dr. Michael Moore
Kim Durham
Alex Costidis
William McLellan

Field # NY4236-2010 *Mn*


NY4236-2010 *Mn* Location on Long Island


NY4236-2010 *Mn* Location in East Hampton

Field # NY4236-2010 *Mn*

| Tissue Held at Bronx Zoo WCS | Whirlpac (# of samples) | Foil (# of samples) | Other (-80) |
|---|---|---|---|
| Heart | 3 | 1 | |
| Lung | 3 | 1 | |
| Liver | 3 | 1 | |
| Bile | | | 1 small nalgene |
| Spleen | 3 | 1 | |
| Kidney | 3 | | |
| Urinary bladder | 3 | | |
| Urine | | | 4 60 ml containers |
| Tongue | 3 | 1 | |
| Baleen 1 | 3 | 1 | |
| Baleen 2 | 3 | 1 | |
| Epiglottic vessicle | | | 1 nunc |
| Pharyngeal mucosa | 3 | | |
| Pancreas | 3 | 1 | |
| Stomach content | | | 1 large nalgene |
| Small intestine | 3 | 1 | |
| Small intestine content | | | 8 3ml vials |
| Large intestine | 3 | 1 | |
| Large intestine content | | | 10 3ml vials |
| Tonsil | 6 | 1 | |
| Lymph node, tracheobronchial | 3 | 1 | |
| Lymph node, mediastinal | 3 | 1 | |
| Lymph node, right retropharyngeal | 3 | 1 | |
| Lymph node, sublumbar | 3 | | |
| Lymph node, mesenteric | 3 | 1 | |
| Lymph node, inguinal | 3 | 1 | |
| Pituitary gland | 1 | | |
| Adrenal gland, right | 3 | 1 | |
| Thyroid gland | 3 | 1 | |
| Skeletal muscle (for UNC) | | | 1 ziplock bag |
| Skeletal muscle, rectus abdominis | 3 | 1 | |
| Diaphragm | 3 | 1 | |
| Conjunctiva, right | 1 | | |
| Conjunctiva, left | 1 | | |
| Retrobulbar tissue, right | 3 | | |
| Retrobulbar tissue, left | 3 | | |
| Vitreous, left eye | 1 | | |
| Vibrissae | 1 | | |
| Skin/blubber, right ventrum | | | 3 ziplock |
| "Abdominal viscera" | 3 | | |
| Abdominal fat | | | 1 large nalgene |
| Cerebrospinal fluid (CSF) | | | 3 3ml vials |
| Brain, cerebrum/cerebellum | | | 2 nunc |
| Ear/Tympanic membrane (glove finger) | 1 | | |
| Copepods | 1 | | |

Field # NY4236-2010 *Mn*

MARINE RESEARCH & PRESERVATION

4/17/2010

| Formalin | Frozen | Serum | Whole Blood | Other |
|---|---|---|---|---|
| Muscle #1 Blubber with skin (8.8cm) | Rt. Ear | Serum 1.0 ml vials (x78) | Whole Blood (EDTA) (x4 vials) DISCARD ? | Ballistics Poss. 2nd deep to blubber (x1) |
| Muscle #1 w/SQ Fat (11.2 cm) | Blubber w/ skin (Ventral 0.5 m) | | | Skin 2x (DMSO) |
| Muscle #1 w/SQ Fat (14.8 cm) | Blubber w/ skin (Ventral 1.0 m) | | | 2 Blowhole cultures in media (awaiting instructions from SR |
| | Chest Fluid (60ml) | | | 2 Blowhole cultures not in media (waiting instructions from SR |
| Ballistics Dorsal Trans. Process Muscle (8.3 cm) | Stovebolt Lft.Back Rostrum | | | |
| Ballistics Dorsal Trans. Process Muscle (5.7 cm) | Stovebolt Rt. Back Rostrum | | | |
| Ballistics Dorsal Trans. Process Muscle (3.9cm) | Stovebolt Right Front Stovebolt | | | |
| Right Flipper Erosion (9.4 cm piece) | Stovebolt Lower Right (2x) | | | |
| Stovebolt Rt.Front Lower | Stovebolt Left Front Rostrum | | | |
| Stovebolt Rt.Front | Muscle Ventral 1.0 m (8.4cm) | | | |
| Stovebolt Front Rostrum | Cyamids (15 ml) | | | |
| Stovebolt Back Rostrum | Blubber Anterior 10m | | | |
| Stovebolt Back Rostrum | Right Eye | | | |
| | Blubber w/Skin (Anterior 0.5m) | | | |
| | Right Flipper Erosions (10.2 cm) | | | |
| | Blubber Caudal (0.5m) | | | |
| | Skin 2x | | | |
| | Muscle Ventral 0.5m (12.5 cm) | | | |
| | Day one injection Site (41cm x 23cm) | | | |
| | Site #2 Injection site (48cm x 28 cm) | | | |
| | Cranial Muscle 0.5 m | | | |
| | Skeletal Muscle Dorsal 0.5m Inj #1 | | | |
| | Muscle Caudal Skeletal 0.5m Inj #2 | | | |
| | Skeletal Muscle Caudal 1.0m Inj #3 | | | |
| | Cranial Muscle SQ Fat 1.0 m | | | |
| | Muscle Needle Inj. Site #2 (24cm x 20cm) | | | |
| | Muscle Needle Inj. Site #1 (21cm x 18cm) | | | |
| | Muscle Injection Site #3 (32cm x 32cm) | | | |
| | Blubber w/Skin Injection Site #3 (72cm x 50cm) | | | |

# APPENDIX 2 -

# HISTOPATHOLOGY REPORT

# NECROPSY REPORT

Field ID: NY4236
Accession Number:10-99C
Species: Megaptera noveangliae
Date: 4/5/10 (DOS); 4/09/10 (DON)
Location: East Hampton, NY (40.9454N, -072.1870W)
Sex, Age Class: M
TL: 939.8 cm
Body Condition:
Condition at Investigation: Alive, Euthanized

## GROSS REPORT

REPORTING INSTITUTION: Riverhead Foundation

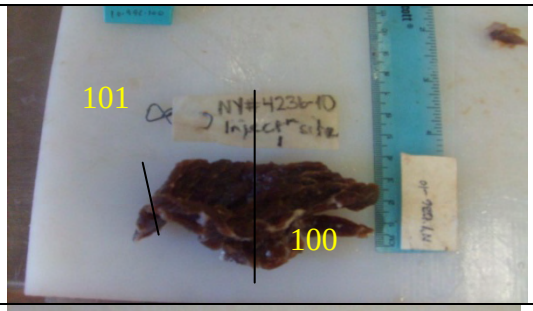
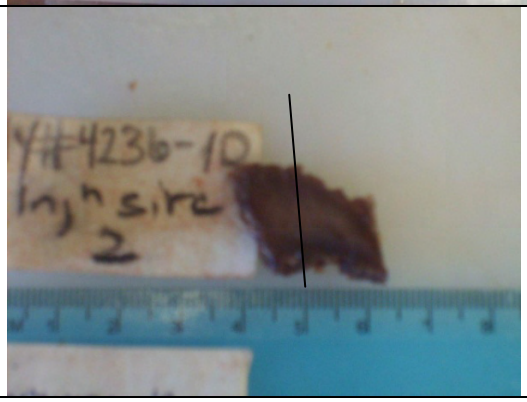
Gross Findings (from gross necropsy report):
1). Carcass: Obesity, marked, generalized
2). Carcass: Possible anemia, generalized (hypovolemic shock)
3). Thoracic cavity, ventral third to half: Fatty deposition, severe, diffuse with caudodorsal displacement and compression of the lung lobes
4). Lymph nodes, multisystemic: Lymphadenopathy/lymphadenomegaly, moderate, multifocal with occasional extracapsular edema fluid and draining hemorrhage
5). Ear, right: Fracture, comminuted, closed, with minimal displacement of bone elements and peri-otic hemorrhage (bullet tract)
6). Brain, right caudal cerebral hemisphere: Hemorrhage, subdural, moderate, focally extensive, acute
7). Brain, meninges, left cerebral hemisphere: Gas bubbles, intravascular, moderate, multifocal to coalescing with scattered perivascular hemorrhage and edema fluid (possible embolization)
8). Fascia, left mandibular body and isthmus: Hemorrhage, subcutaneous and fascial, marked, focally extensive, acute
9). Abdominal wall, right ventral: Cellulitis, moderate, focal, fibrinonecrotizing, with multifocal myocellular degeneration and necrosis of the adjoining abdominal rectus musculature and necrosis of the overlying blubber
10). Skin, midventral abdomen: Constriction bands, moderate, bifocal, linear (possible human interaction)
11). Kidney: Nematodiasis, encapsulated, mild, focal, subacute
12). Subcutis, proximal, midventral peduncle: Hematoma, longitudinal, moderate, focal, acute
13). Stomach, nonglandular compartment: Pica, moderate, focal (ingested sand)
14. Ductus arteriosus: Contracted but probe patent.

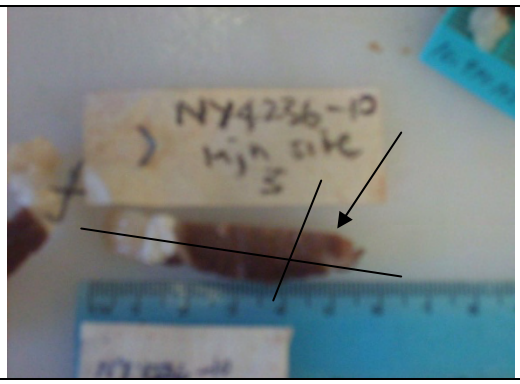
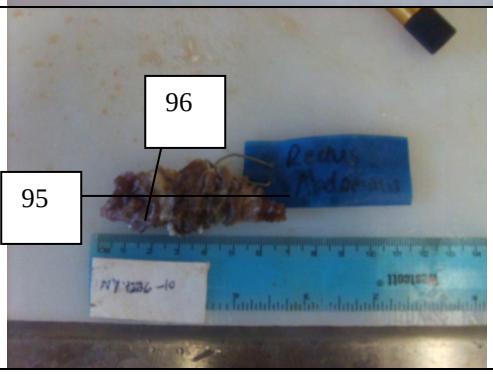
## ANCILLARY FINDINGS

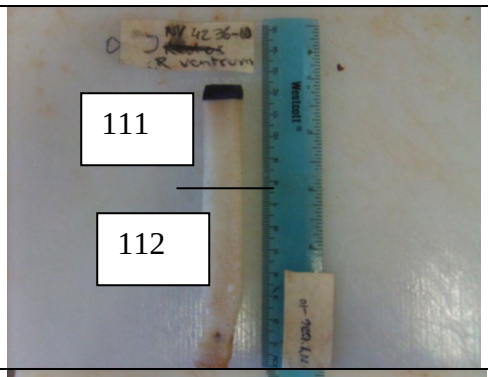
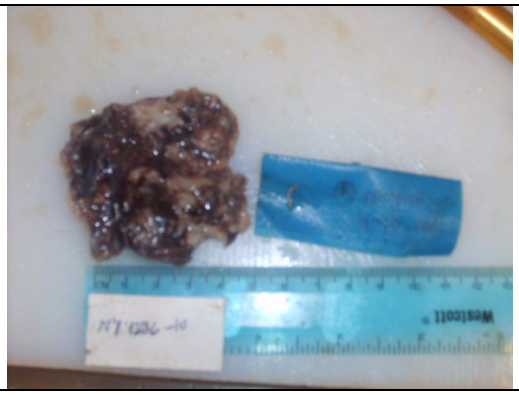
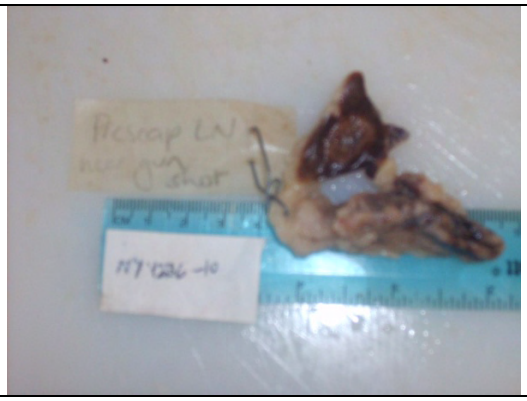
| Test | Tissue Tested | Result | Comment |
|---|---|---|---|
| CBC, Chemistry Panel | Blood | Hypokalemia; hypophosphatemia; hypercalcemia; | |
| Urinalysis | Urine | No significant findings. | |
| MRI | Brain | Pending | MRI @ Mt. Sinai; Radiologist-Dennison |
| Urine Myoglobin | Urine | Negative | Texas Vet Med Diag Lab |
| Renal Parasite | Parasite | Crassicauda boopis | Cornell and Sea Rogers Williams |
| | | | |

## TISSUES/SAMPLES RECEIVED

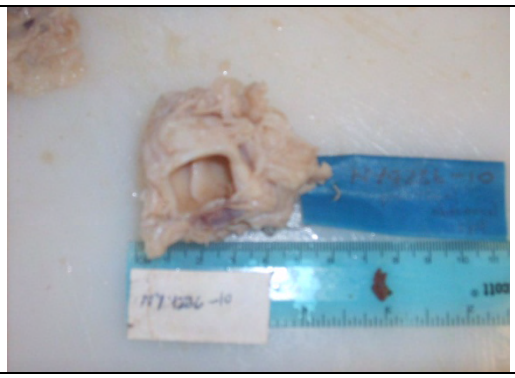
Received 17 April 2010 is a box containing 4 large containers and 1 small vial of a renal nematode in EtOH. The 4 large containers contain 56 labeled and 33 unlabeled formalin fixed tissues. Disposition of these samples is listed in Table 1 below. Representative sections of all samples are placed in Cassettes 1-112. The parasite in EtOH was submitted to Dr. Sea Rogers Williams at the National Marine Life Center, Buzzards Bay, Maine.

| Tissue Sample; size (if applies) | Labeled (X) | Cassette | Photo (Y/N) | Sampling Site |
|---|---|---|---|---|
| Skeletal muscle-injection site 1<br>9 X 3 X2.5 cm | X | 100, 101 | Y |  |
| Skeletal muscle-injection site 2<br>3 X 2 X 2 | X | 106 | Y |  |

| | | | | |
|---|---|---|---|---|
| Skeletal muscle-injection 3 5.5 X 2 X 1; note pallor of muscle (arrow) | X | 102, 103 | Y |  |
| Skeletal muscle-injection 3 12.5 X 2 X 1 | X | 104, 105 | N | |
| Skeletal muscle-right retrobulbar | X | 94 | N | |
| Skeletal muscle-right ventrum | X | 63 | Y |  |
| Skeletal muscle-caudal abdomen | X | 97-99 | Y |  |
| Skeletal muscle-rectus abdominis | X | 95, 96 | Y |   |

| | | | | |
|---|---|---|---|---|
| Diaphragm | X | 56 | N | |
| Skin/blubber-right ventrum | X | 111, 112 | Y |   |
| Skin/blubber-mandibular symphysis | X | 107-110 | Y |  |
| Vibrissae | X | 36 | N | |
| Tonsil | X | 23, 49 | N | |
| Lymph Node-right retropharyngeal | X | 43 | Y |  |
| Lymph Node-mediastinal | X | 39, 40 | N | |
| Lymph Node-prescapular near gunshot | X | 58, 59 | Y |  |

| | | | | |
|---|---|---|---|---|
| Lymph Node-inguinal | X | 79 | N | |
| Lymph Node-sublumbar | X | 18, 19 | N | |
| Lymph Node-tracheobronchial | X | 40 | N | |
| Lymph Node-mesenteric | X | 89 | N | |
| Splenic choristoma | X | 44 | N | |
| Aorta | X | 86 | N | |
| Foramen ovale | X | 84, 85 | N | |
| Larynx mm | X | 3 | N | |
| Larynx | X | 22, 22 | N | |
| Lung-apical | X | 43 | N | |
| Lung-hilar | X | 83 | N | |
| Lung-middle | X | 20 | N | |
| Baleen (1, 2) | X | 26 | N | |
| Tongue | X | 13-15 | N | |
| Pharyngeal Ulcer | X | 37, 38 | N | |
| Periepiglottic vesicle | X | 46 | N | |
| Left epiglotic erosion | X | 42 | N | |
| Right epiglotic erosion | X | 52 | N | |
| Stomach-Jct C1/C2 | X | 24, 25 | N | |
| Stomach-C2 | X | 34, 35 | N | |
| Stomach-C3/C4 | X | 16 | N | |
| Small intestine-lesion | X | 1, 2 | N | |
| Pancreas(??) | X | 41 | N | |
| Pancreas | X | 91 | N | |
| Kidney-right | X | 75 | N | |
| Kidney-left | X | 92 | N | |
| Ureter | X | 55 | N | |
| Urinary Bladder | X | 78 | N | |

| | | | | |
|---|---|---|---|---|
| Perirenal abdominal Cyst | X | 73 | Y |  |
| Retroperitoneal Abscess-Left Kidney | X | 32 | N | |
| Thyroid Gland | X | 82 | N | |
| Adrenal Gland-right | X | 45 | N | |
| Pituitary Gland | X | 51 | N | |
| Testicle right | X | 46 | N | |
| Conjunctiva-right | X | 60 | N | |
| Conjunctiva-left | X | 4 | N | |
| Cauda equine | X | 23 | N | |
| Heart | -- | 7-10, 27a, 47, 48, 53, 54 | N | |
| Lung | -- | 27, 28, 31, 69 | N | |
| Liver | -- | 5, 58, 64, 68 | N | |
| Spleen | -- | 6, 30 | N | |
| Kidney | -- | 17, 61, 62, 72, 74, 77, 81 | N | |
| Trachea | -- | 67 | N | |
| Gastrointestinal | -- | 29, 65, 66, 80, 87, 88 | N | |
| Penis | -- | 11 | N | |
| Brainstem/cord | -- | 70, 71 | N | |

4/29/2010

Drs. Patrick Hof (Mount Sinai School of Medicine) and Rotstein examined and subsampled the brain at Mount Sinai. Photographs of the samples and the

process as well as a copy of the MRI were received by D. Rotstein. Samples were labeled and shipped via Fed Ex to D. Rotstein.

Samples include the following all collected from the right side:

| Brain Location | Histo Cassette-Regular (R) or MegaCassette (M) | Additional Sample | Cassette Letter | Processing: HE, oil red O (O) |
|---|---|---|---|---|
| Temporal Lobe | M | Yes | A | HE |
| Parietal Lobe | R | Yes | B | HE |
| Hippocampus | M | Yes | C | HE |
| Pituitary Gland | M | Yes | C | HE |
| Occipital Lobe | M | Yes | D | HE |
| Basal Ganglia | M | Yes | D | HE |
| Rete Miriable | R | Yes | E | HE, O |
| Cortex (frontal, slab 11) | M | Yes | F | HE |
| Thalamus | M | Yes | F | HE |
| Choroid Plexus | R | Yes | G | HE |
| Spinal Cord | R | Yes | H | HE, O |
| Frontal Lobe | M | Yes | I | HE |
| Vermis and Cerebellum | M | Yes | J | HE |
| Lateral Cerebellum | M | Yes | K | HE |
| Mesencephalon | M | Yes | L | HE |
| Lower Bulb | M | NO | M | HE |
| Pontine Nuclei | M | Yes | N | HE |
| Right Occipital Lobe (site of subdural hemorrhage) | M | Yes | O | HE |

## MICROSCOPIC FINDINGS

Digestive System Suite

Slides 1-2

Intestine: Within the tunica submucosa and muscularis flanking vessels, there are scattered to aggregated eosinophils,neutrophils, lymphocytes, and plasma cells (Figure 1). The tunica serosa is expanded by extravasated erythrocytes and moderate numbers of eosinophils and macrophages. Macrophages occasionally contain nuclear debris (phagocytosis) (Figure 2). There is thickening (fibrosis) of the tunica serosa. There is congestion of small and medium-sized vessels within the all layers.

Slide 16
Stomach-Junction C3/C4: There is a sprinkling of plasma cells and eosinophils within the submucosa.

Slides 24, 25
Stomach-Junction C1/C2: Three (3) sections are examined. Within the submucosa, there are multifocal to coalescing infiltrates of neutrophils and fewer lymphocytes, plasma cells, and macrophages.

Slides 34, 35
Stomach C2: Two (2) sections are examined. Plasma cells, lymphocytes, and neutrophils expand the mucosa. Mucosal-assocaited lymphoid tissues are prominent. Within the submucosa and serosa, there are multifocal to coalescing infiltrates of eosinophils, plasma cells, and lymphocytes. Inflammatory cells track around vessels. Vessels exhibit medial thickening.

Slides 41, 91
Pancreas: Four (4) sections are examined. There is depletion of zymogen granules. Within the peripancreatic adipose, there is a severe, nearly diffuse infiltrate of primarily neutrophils, fewer eosinophils and macrophages (Figure 3) forming loose to dense aggregates or coalescing into eosinophilic granulomas (Figure 4). Eosinophils typically tract along adipocyte membrane borders and there is minimal adipocyte necrosis. Macrophages contain intracytoplasmic nuclear debris or brown granules (hemosiderin vs ceroid-lipofuscin). There is multifocal fibroplasia characterized by aggregates of plump fibroblasts within a collagenous matrix with interspersed plump-endothelial lined vascular channels. There is multifocal hemorrhage.

Slides 29, 65, 66, 80, 87
Stomach and Intestine, NOS: Nine (9) sections are examined. In the stomach (C3/C4), there is a mild increase in mucosal lymphocytes, plasma cells, and eosinophils with occasional regions of glandular loss and replacement by fibrous connective tissue. Within the submucosa, there are occasional granulomata composed of epithelioid macrophages with interspersed eosinophils, lymphocytes, and plasma cells. In the intestine, there are increased eosinophils and plasma cells within the lamina propria. Hemosiderin-laden macrophages are scattered throughout. Eosinophils and macrophages were present in variable numbers within the serosa of most sections.

Slide 88
Mesentery: There are multifocal to coalescing infiltrates of eosinophils with fewer macrophages, plasma cells, and lymphocytes within the adipose, supporting collagenous stroma, and around vessels. Vessels exhibit medial thickening and occasional venules contain segmental mural thrombi which protrude into the lumen and are covered by endothelium (Figure 5).

Ocular System Suite

Slide 4
Conjunctiva, Right: No Significant Histologic Findings (NSF).

Slide 60
Conjunctiva, Left: NSF.

Slide 12
Optic Nerve, Left Eye: Three sections are examined. NSF.

Cardiovascular System Suite

Slides 7-9, 27a, 47, 48, 53, 54
Heart: Fifteen sections are examined. Rarely within the coronary groove fat, there are adipocytes which contain few foamy macrophages (Figure 6).

Slide 10
Pulmonary Artery: NSF.

Slides 84, 85
Foramen Ovale: Two (2) sections are examined. There is extensive fibrosis.

Slide 86
Aorta: NSF.

Hepatobiliary System Suite

Slides 5, 50, 64, 68
Liver: Seven (7) sections are examined. Diffusely, hepatocytes have a swollen, eosinophilic diaphanous cytoplasm or vacuolated cytoplasm (Figure 7). Occasional bile ducts contain grey-green wispy to granular material (bile). In one section (Slide 68), there is portal fibrosis and a mild increase in periportal lymphocytes and plasma cells. Brown-black granules (hemosiderin) is present within the cytoplasm of random Kupffer cells.

Genitourinary System Suite

Slides 17, 61, 62, 72, 74, 81
Kidney, NOS: Ten (10) sections composed of 33 partial and complete reniculi are examined. There are random reniculi with distended proximal tubules lined by mildly attenuated epithelium. Within the majority of proximal tubules, there are

lightly basophilic globular to lightly eosinophilic wispy substance. There is peripelvic fibrosis and peripelvic edema.

Slide 75
Kidney, Right and Proximal Ureter: A complete reniculus, 3 partial reniculi, and ureter are examined. Proximal tubules contain globular, basophilic substance. In a reniculus, there is mild, multifocal interstitial fibrosis with associated tubular loss(Figure 8). Within the ureter, there are submucosal eosinophilic and plasmacytic infiltrates. There is arterial medial hypertrophy with mild diminutization of vascular lumina. The endothelium is thrown in small folds due to an increased subintimal vacuolated stroma.

Slide 92
Kidney, Left and Proximal Ureter: Three reniculi are examined. In a reniculus, there is mild, multifocal interstitial fibrosis with associated tubular loss and glomerulosclerosis. Tubules contain smudgy eosinophilic to basophilic globular substance. In the ureter, there is submucosal edema (Figures 9a and 9b) and multifocal loosely cellular to nodular infiltration by lymphocytes, plasma cells, and fewer eosinophils.

Slide 73
Perirenal Abdominal Cyst: Two (2) sections are examined. There is a dense band of collagen with interspersed mineral (dystrophic mineralization), eosinophils, macrophages, and plasma cells. At one edge, there are entrapped adipocytes within the fibrous stroma.

Slide 32
Retroperitoneal Abscess-Left Kidney: Two (2) sections are examined. There is a thick fibrous capsule interdigitating with adipose (Figure 10). Within the region of fibrosis, there are moderate numbers of plasma cells, macrophages, lymphocytes, and multinucleated giant cells (Figure 11). Light brown granules are present freely within the inflammatory cell infiltrate or within macrophages. There is multifocal adipocyte necrosis.

Slide 55
Ureter: Two (2) sections are examined. Within the periureteral adipose, there are coalescing infiltrates of plasma cells, macrophages, and eosinophils within a fibrotic stroma (Figure 12). Light brown granules are present freely within the inflammatory cell infiltrate or within macrophages. Within regions of fibrosis, there are deposits of magenta mineral (Figure 12).

Slide 78
Urinary Bladder: Three (3) sections are examined. NSF.

Slide 76

Testis: Two (2) sections are examined. Seminiferous tubules are lined by a single layer of germ cells. There is rare and very minimal development of spermatagonia.

Slide 11
Penis: Two (2) sections are examined. NSF.

Lymphoreticular System:

Slides 6 and 30 (Spleen) and 44 (Splenic Choristoma)
Spleen and Splenic Choristoma: Four (4) sections are examined. The spleen and splenic choristoma have moderately cellular periarteriolar lymphoid sheaths and overall a similar architecture. Within the spleen, there are occasional, small islands of lymphocytes separated by a fibrous stroma near the capsule. There is moderate congestion.

Slides 18, 19
Lymph Node, Sublumbar: Two (2) sections are examined. Lymphoid follicles are well-developed and highly cellular. There are increased eosinophils within medullary cords. Within the medullary sinus, there are moderate numbers of erythrocytes and macrophages. Occasional macrophages contain intracytoplasmic erythrocytes (erythrophagocytosis).

Slides 39, 40
Lymph Node, Mediastinal: Three (3) sections are examined. Findings are similar to Slides 18 and 19.

Slide 57
Diaphragmatic Lymph Node: Findings are similar to Slides 18 and 19.

Diaphragm: Two sections are examined. NSF.

Slides 58, 59
Lymph Node, Prescapular, Near Gunshot: Two (2) sections are examined. There is marked congestion of the lymph node with medullary spaces expanded by erythrocytes. Lymphoid follicles are well-developed. Within the perinodal adipose, there is extensive extravasation of erythrocytes (Figure 13).

Slide 79
Lymph Node, Inguinal: Findings are similar to Slides 18 and 19.

Slides 89
Lymph Node: Mesenteric: Two (2) sections are examined. There is moderate lymphoid hyperplasia. Eosinophils are increased in number and form loosely

aggregated nodules. There are segmental perivascular accumulations of eosinophils.

Slide 90
Lymph Node, Tracheobronchial: Two (2) sections are examined. Findings are similar to Slides 18 and 19.

Slide 93
Lymph Node, Retropharyngeal: Two (2) sections are examined. Findings are similar to Slides 18 and 19.

Ororespiratory Suite

Slide 3
Larynx-Skeletal Muscle: Two (2) sections are examined. No significant histologic findings (NSF).

Slides 13-15
Tongue: Four (4) sections are examined. NSF.

Slides 21, 22
Larynx: Four (4) sections are examined. Stromal vascular endomthelial cells are plump (reactive).

Slide 20
Lung-Middle: Two (2) sections are examined. There are occasional bronchioles and rare alveolar lumina which contain neutrophils, macrophages, and eosinophils within a mucoproteinaceous exudate. There are occasional coccoid bacteria. There is bronchiolar submucosal infiltration by lymphocytes, plasma cells, and eosinophils with eosinophilic transcytosis.

Slide 43
Lung-Apical: Two (2) sections are examined. There is bronchiolar submucosal infiltration by lymphocytes, plasma cells, and eosinophils with eosinophilic transcytosis.

Slide 83
Lung-Hilar: There are occasional bronchioles and rare alveolar lumina which contain neutrophils, macrophages, and eosinophils within a mucoproteinaceous exudate. Bacterial cocci are present in small numbers within inflammatory foci. There is bronchiolar submucosal infiltration by lymphocytes, plasma cells, and eosinophils.

Slides 27, 28, 31, 69
Lung, NOS: Eight (8) sections are examined. Within occasional bronchioles, there are neutrophils, macrophages, and eosinophils within a matrix of

mucoproteinaceous exudate (Figures 14, 15). There are lymphocytes, plasma cells, and eosinophils within the bronchiolar submucosa. There is random collapse of alveoli and increased cellularity (artifact). Within occasional alveoli, there are few foamy macrophages. Occasional vessels are infiltrated by eosinophils (Figure 16).

Slide 67
Trachea: Minimal respiratory mucosal epithelium is present. There are small numbers of plasma cells and lymphocytes within the submucosa.

Slides 23, 49
Tonsil: Four (4) sections are examined. There is a focal mucosal epithelial ulceration with associated neutrophils.

Slides 37, 38
Pharyngeal Ulcer: Four (4) sections are examined. The section consists of a variably-dense stratified squamous epithelium that is invaginated and flanked by prominent lymphoid follicles. In one section, there are submucosal glands (Slide 38).

Slide 42
Left Epiglotic Erosion: There is an approximately 4.0 mm ulceration of the mucosal epithelium (Figure 17). Viable and degenerate neutrophils cover the defect and extend into the submucosa. There are bacterial cocci forming small, dense clumps within the necrotic epithelium. There are macrophages and few, plump streaming fibroblasts within the submucosa. Vessels within this region are lined by plump endothelial cells (fibroplasia). Within the adipose, there is a nodular focus of fibroblasts with abundant myxomatous cytoplasm. At one edge, there is focally extensive mucosal epithelial hyperplasia with arborizing and interdigitating epithelial fronds extending into the submucosa.

Slide 52
Right Epiglotic Erosion: Two (2) sections are examined. Findings are similar to Slide 42.

Slide 46
Periepiglotic Vesicle: The section consists of a variably-dense stratified squamous epithelium that is invaginated and flanked by prominent lymphoid follicles and glands.

Slide 26
Baleen: NSF.

Nervous System Suite

Slide 33
Cauda Equina: NSF.

Slides 70 and 71
Spinal Cord: Four (4) sections are examined. NSF.

| Brain Location | Cassette Letter | Processing: HE, oil red O (O) | Histologic Findings |
|---|---|---|---|
| Temporal Lobe | A | HE | Hemorrhage, perivascular, multifocal, acute, minimal. |
| Parietal Lobe | B | HE | Mild, multifocal, satellitosis<br>Vascular Congestion |
| Hippocampus | C | HE | NSF |
| Pituitary Gland | C | HE | NSF |
| Occipital Lobe | D | HE | Hemorrhage, meningeal, acute, multifocal, mild. |
| Basal Ganglia | D | HE | NSF |
| Rete Miriable | E | HE, O | NSF; No fat present in vascular lumina. |
| Cortex (frontal, slab 11) | F | HE | Hemorrhage, perivascular, multifocal, acute, minimal. |
| Thalamus | F | HE | Hemorrhage, perivascular, multifocal, acute, minimal. |
| Choroid Plexus | G | HE | NSF |
| Spinal Cord | H | HE, O | NSF; No fat present |
| Frontal Lobe | I | HE | Hemorrhage, perivacular, multifocal, acute, minimal. |
| Vermis and Cerebellum | J | HE | 1.Vacuoles, cytoplasmic, Purkinje cell, multifocal, minimal.<br>2.Hemorrhage, meningeal and perivacular, multifocal, acute, minimal. |
| Lateral Cerebellum | K | HE | Hemorrhage, perivacular, multifocal, acute, minimal. |
| Mesencephalon | L | HE | Hemorrhage, perivacular, multifocal, acute, minimal. |
| Lower Bulb | M | HE | Hemorrhage, perivacular, multifocal, acute, minimal. |
| Pontine Nuclei | N | HE | Hemorrhage, perivacular, multifocal, acute, minimal. |
| Occipital Lobe (site of subdural hemorrhage) | O | HE | 1.Subdural and superficial cortical hemorrhage, acute, moderate (Figures 23 and 24).<br>2.Vascular congestion and perivascular hemorrhage. |

Integumentary/Musculoskeletal System Suite

Slide 36
Vibrissae: Elongate bacteria are present on the surface.

Slide 63
Skin/Blubber, Right Ventrum: Two (2) sections are examined. The section consists of myofibers in cross section, adipocytes with interspersed collagen bundles. There is multfiocal collagenolysis with associated deposition of basophilic stippled to angular substance (dystrophic mineralization) (Figure 18, 19).

Slide 94
Skeletal Muscle, Retrobulbar: Two (2) sections are examined. NSF.

Slides 95, 96
Skeletal Muscle, Rectus abdominis: Three (3) sections are examined. There is multifocal basophilia and granularity of collagen bundles and muscle. In one section (slide 96), there is eosinophilic granular substance within the fibroadipose.

Slides 97-99
Skeletal Muscle, Caudal Abdomen: Four (4) sections are examined. There are random individual myofibers which are degenerate and have associated neutrophils and rare eosinophils. Affected myofibers account for less than 1% of the examined myofibers. Within the fibroadipose, there are occasional foci of macrophages, plasma cells, and rare eosinophils.

Slides 100, 101
Skeletal Muscle, Injection Site 1: Three (3) sections are examined. NSF.

Slides 102, 103
Skeletal Muscle, Injection Site 3 Number 1: Four (4) sections are examined. In two sections, there is a sharp demarcation between intact myofibers with distinct borders to myofibers that are diffusely granular with nearly imperceptible cell borders (Figure 20). There is no associated extravasation of erythrocytes or inflammatory cells.

Slides 104, 105
Skeletal Muscle, Injection Site 3 Number 2: Three (3) sections are examined. Findings are similar to Slides 102 and 103.

Slide 106
Skeletal Muscle, Injection Site 2: NSF.

Slides 107-110

Mandibular Symphysis: Five (5) sections are examined. There is an abrupt transition from small islands of bone with interspersed fibrocytes to dense sheets of plump fibroblasts with intercellular collagen deposition (Figures 21, 22)(periosteunm). At the edge of the fibroblastic response, there are a few vessels with a sprinkling of perivascular lymphocytes and plasma cells. In one section (slide 108), there is a tract of extravasated erythrocytes, fewer neutrophils, and fibrin. There is hyperplasia of the overlying epithelium and within the reticular dermis, there are small foci of dermal fibroplasia.

Slides 111, 112
Skeletal Muscle, Right Ventrum: Three (3) sections are examined. The sections consist of epithelium and fibroadipose. There are few perivascular lymphocytes and plasma cells within the reticular dermis.

## Endocrine System Suite

Slide 45
Adrenal Gland, Right: Two (2) sections are examined. NSF.

Slide 51
Pituitary Gland: The anterior pituitary is present. NSF.

Slide 82
Thyroid Gland: Two (2) sections are examined. Follicles are lined by a uniform layer of cuboidal cells and contain abundant, homogenous brightly eosinophilic colloid.

## FINAL DIAGNOSES/INTERPRETATIVE SUMMARY

Digestive System/Abdominal Cavity:

A.Mesentery: Mesenteric steatitis, eosinophilic to granulomatous, multifocal to coalescing, severe with segmental vascular thrombosis and endothelial proliferation.

B.Stomach and Intestine,Submucosa, Muscularis, and Serosa:

1.Gastroenteritis, neutrophilic and histiocytic, multifocal to coalescing, moderate, chronic-active.
2.Serosal fibrosis.

C.Intestine: Enteritis, plasmacytic and eosinophilic, multifocal, minimal.

D.Pancreas: Peripancreatic steatitis, eosinophilic to granulomatous, multifocal to coalescing, severe with fibroplasia.

Urogenital System:

A.Retroperitoneal and Perirenal Abdominal Pseudocysts: Fibrosis, granulomatous celllulitis, and mineralization.

B.Ureter:

1.Proximal: Ureteritis, lymphoplasmacytic and eosinophilic with edema.

2.Distal: Periureteritis (cellulitis), lymphoplasmacytic, histiocytic, and eosinophilic with fibrosis and multifocal dystrophic mineralization.

C. Kidney:

1.Tubular loss and fibrosis, multifocal, mild.

2.Tubular distention with tubular epithelial attenuation, multifocal, mild.

D.Testis: Inactive (aspermatogenesis).

Hepatobiliary System:

Liver:Mixed vacuolar hepatopathy (glycogen accumulation and lipid accumulation).

Lymphoreticular System:

A.Lymph Nodes, Multiple (Retropharyngeal, Tracheobronchial, Mesenteric, Prescapular, Inguinal, Diaphragmatic, Mediastinal, Sublumbar):

1.Lymphoid hyperplasia, moderate.

2.Draining lymph node (erythrocytes).

3.Eosinophilia.

B.Prescapular Lymph Node (Near Site of Gunshot): Perinodal hemorrhage.

Respiratory System:

A.Lung:

1.Bronchiolotis, neutrophilic and histiocytic, acute, multifocal, minimal to mild with minimal lumneal exudates (neutrophils, macrophages, eosinophils, and mucus) and rare inalumenal bacteria (coccoid).

2.Periarteritis and arteritis, eosinophilic, multifocal, mild.

B.Left and Right Epiglottis: Ulcerative epiglottits, suppurative with fibroplasia and regionally extensive epithelial hyperplasia.

Nervous System:

Occipital Lobe, Cerebrum: Hemorrhage, subdural and parenchymal, focally extensive, moderate.

Integumentary/Musuloskeletal System:

A.Right Ventrum: Collagenolysis and dystrophic mineralization.
B.Rectus abdominis: Collagenolysis and dystrophic mineralization.
C.Caudal Abdomen: Myofiber degeneration and neutrophilic myositis, multifocal, mild.
D.Injection Site 3: Myofiber degeneration (medicinal-associated).
E.Mandibular Symphysis:
1. Hemorrhage and fibrin exudation.
2.Mild periosteal proliferation.

Cardiovascular System:
A.Foramen Ovale: Fibrosis.
B.Coronary Fat: Histiocytic steatitis, mild, multifocal.

| Diagnosis |
|---|
| Euthanasia |
| Eosinophilic Peripancreatitis, Serositis, Mesenteric Steatitis |
| Retroperitoneal and Renal Parasitic Pseudocyst |
| Suppurative Bronchiolitis |
| Draining Lymph Node (Congestion) |
| Collagen Mineralization (Rectus Abdominis) |
| Steroid Hepatopathy |
| Subdural and Parenchymal Hemorrhage-Left Occipital Lobe |

A male humpback whale stranded in East Hampton, New York and was euthanized. Euthanasia involved chemicals and ballistics.

A cause or contributing cause of stranding associated with significant peracute, acute, or chronic inflammatory or infectious processes was not observed with microscopic examination of tissues. There was gross and histologic evidence of renal and perirenal infiltration by Crassicauda sp., a nematode. This nematode is not uncommon in large whales and has been observed in fin whales, blue whales, and humpback whales as well as other species. There is variability in severity and the impact upon whales is not completely known (Raga et al. 1997. Parasitologia 39: 293-296). In this humpback whale, there were granulomas present, but the majority of the kidney was not affected; clinical chemistry results did not indicate renal dysfunction.

There was glycogen and lipid present within hepatocytes. The glycogen may represent a “steroid hepatopathy”, that is increased cortisol. Cortisol can be produced as a stress response and an increase in a stranding of any cetacean would not be unexpected. The presence of lipid likely indicates mobilization of body fat stores if an animal has not been eating.

In the ventrum involving the collagen sheath and muscle, there was damaged collagen and skeletal muscle fibers (myofibers) which was likely the result of

compression on these soft tissues from a large animal that normally is within the water column with less pressure on the abdomen to an animal on land with significantly greater pressure on the abdomen. Muscle necrosis was also observed at injection sites. This is the result of the administered chemicals and is an expected response. Muscle necrosis leads to release of myoglobin from damaged muscles. In large amounts, myoglobin can cause damage to renal tubules. This condition is known as “myoglobinuric nephrosis”. In addition to the microscopically evident changes, there is an increase in creatine phosphokinase (CPK, CK). These changes were NOT observed in this humpback whale.

Near the site of ballistic usage, there was drainage of blood in the prescapular lymph node. In the left occipital lobe, there was focal sudural hemorrhage and hemorrhage within the neuropil. As no bullet fragments or tract was observed that penetrated the skull, this hemorrhage is likely the result of concussive forces from the ballistic.

The bronchiolitis is acute and likely reflects diminished pulmonary clearance. Gastroenteritis may reflect a response to parasites.

Grossly an intrathoracic accumulation of adipose was present. Histologically, it was composed of mature adipocytes with no evidence of inflammation or necrosis. This fat body could represent a normal accumulation of adipose in a well-conditioned animal. The affect of this fat body on cardiac and respiratory function in a stranded cetacean is not known.

## FIGURES

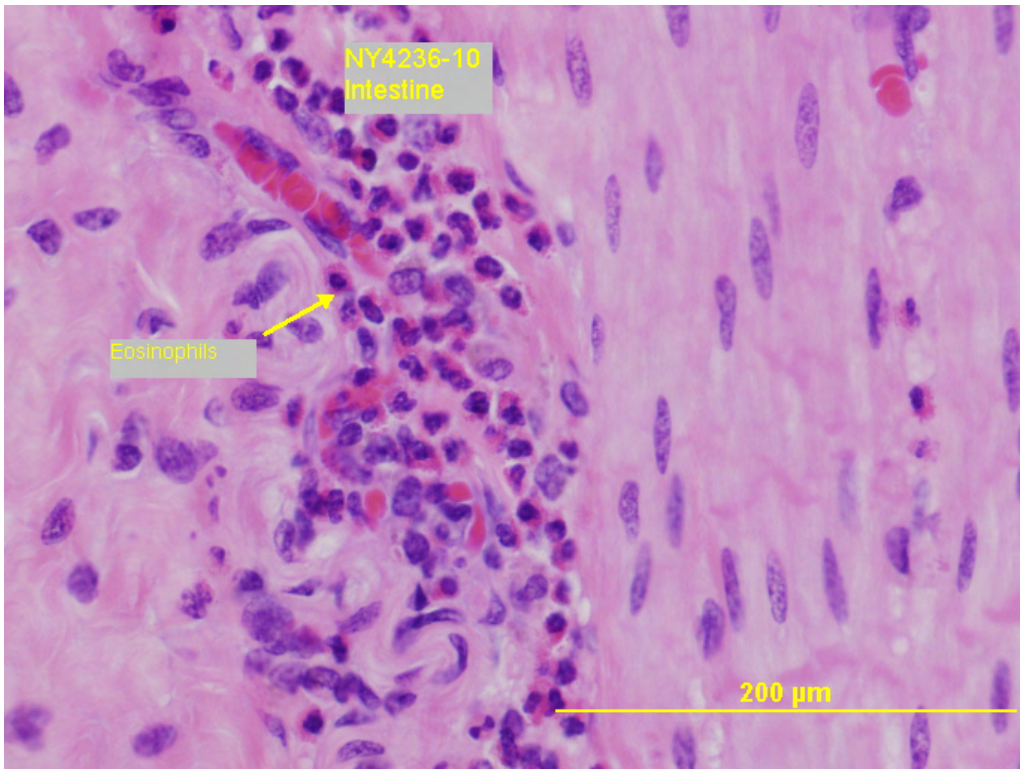



Figure 1. Intestine. Eosinophils and plasma cells flank vessels and are sprinkled with the tunica submucosa.

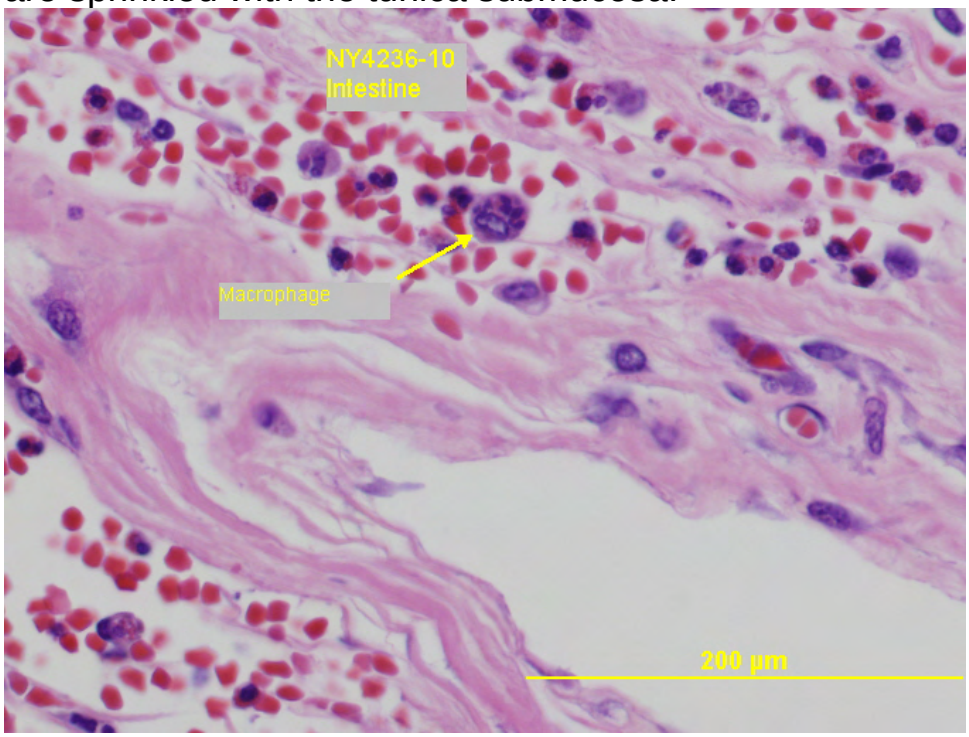



Figure 2. Intestine: Macrophages contain abundant nuclear debris (phagocytosis).

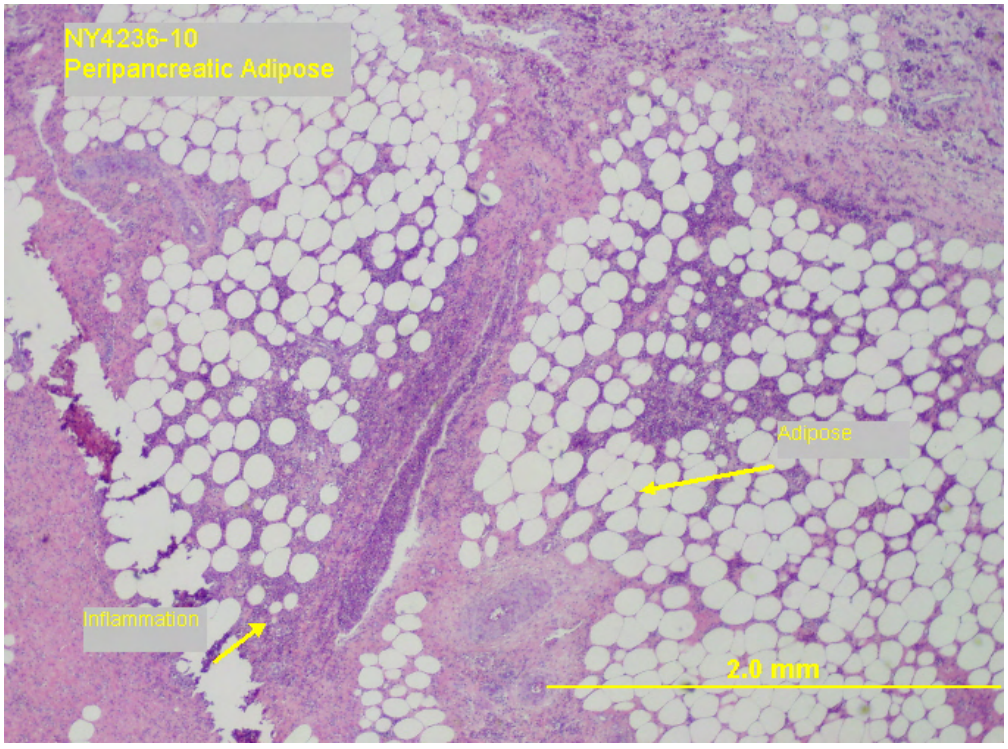



Figure 3. In the peripancreatic adipose, there are large numbers of eosinophils.

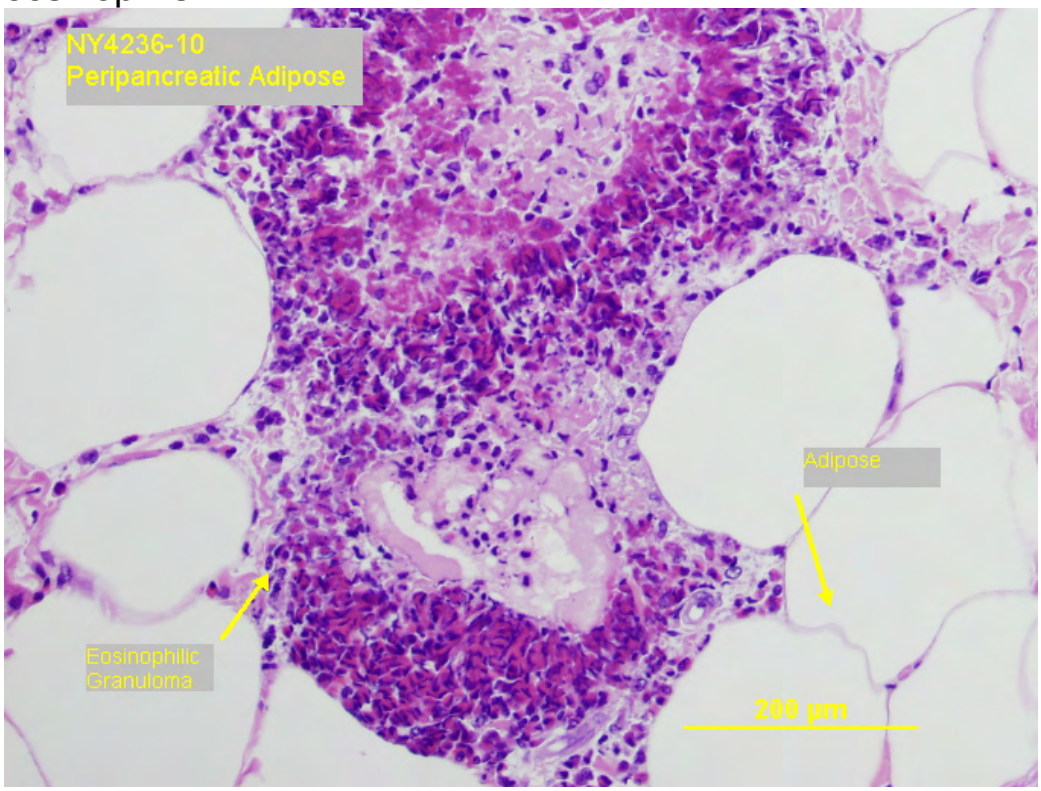



Figure 4.Pancreas. Within the peripancreatic adipose, there are eosinophilic granulomas.

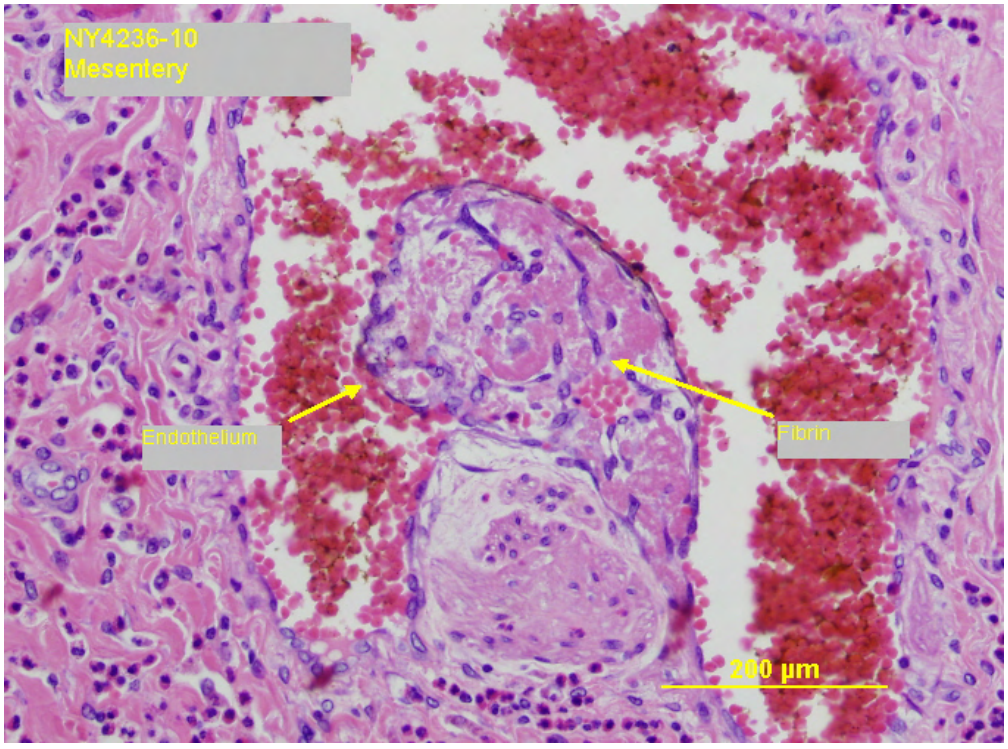



Figure 5. Mesentery-Vein. There is protrusion of an endothelium covering a mass of fibrin and few fibroblasts.

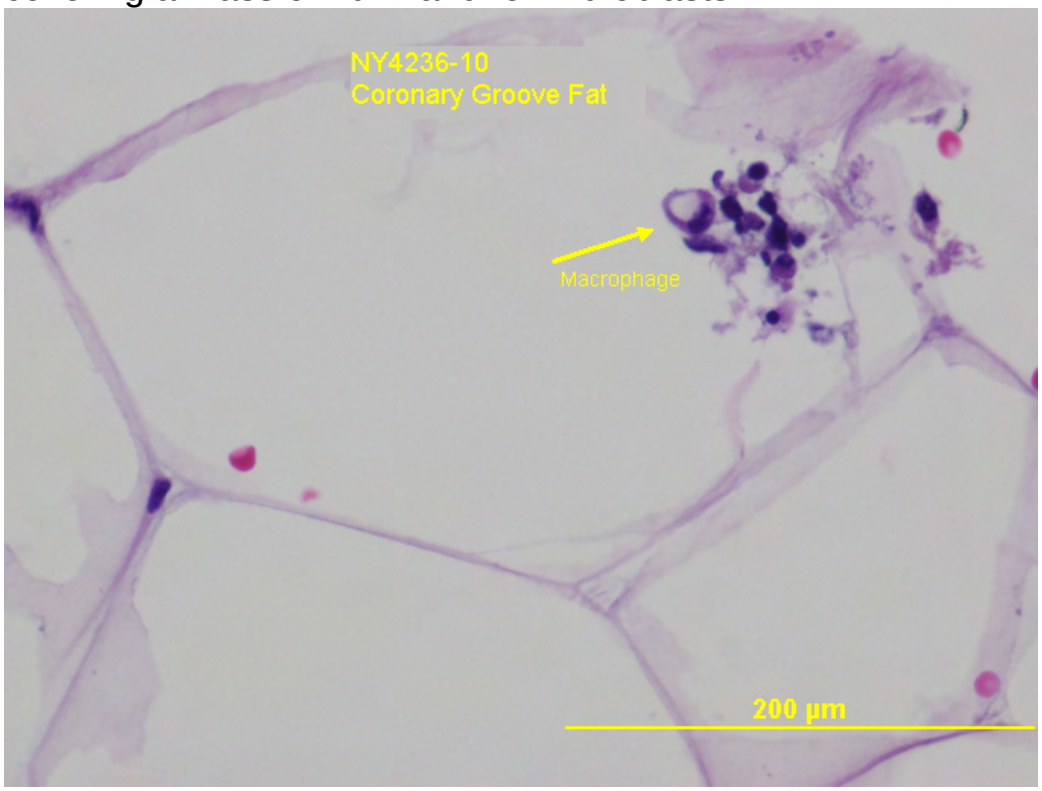
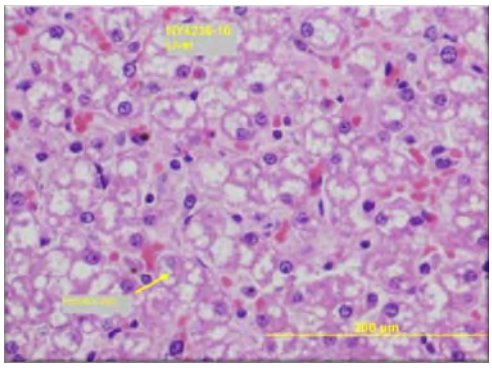
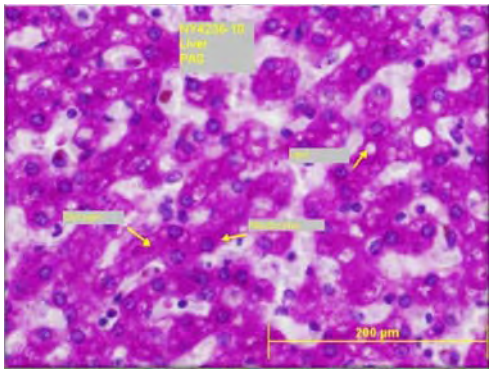



Figure 6. Heart, Coronary Groove. Occasional adipocytes contain foamy macrophages.





Figures 7a and 7b. Liver. Hepatocytes are swollen and have a wispy eosinophilic cytoplasm (glycogen accumulation).

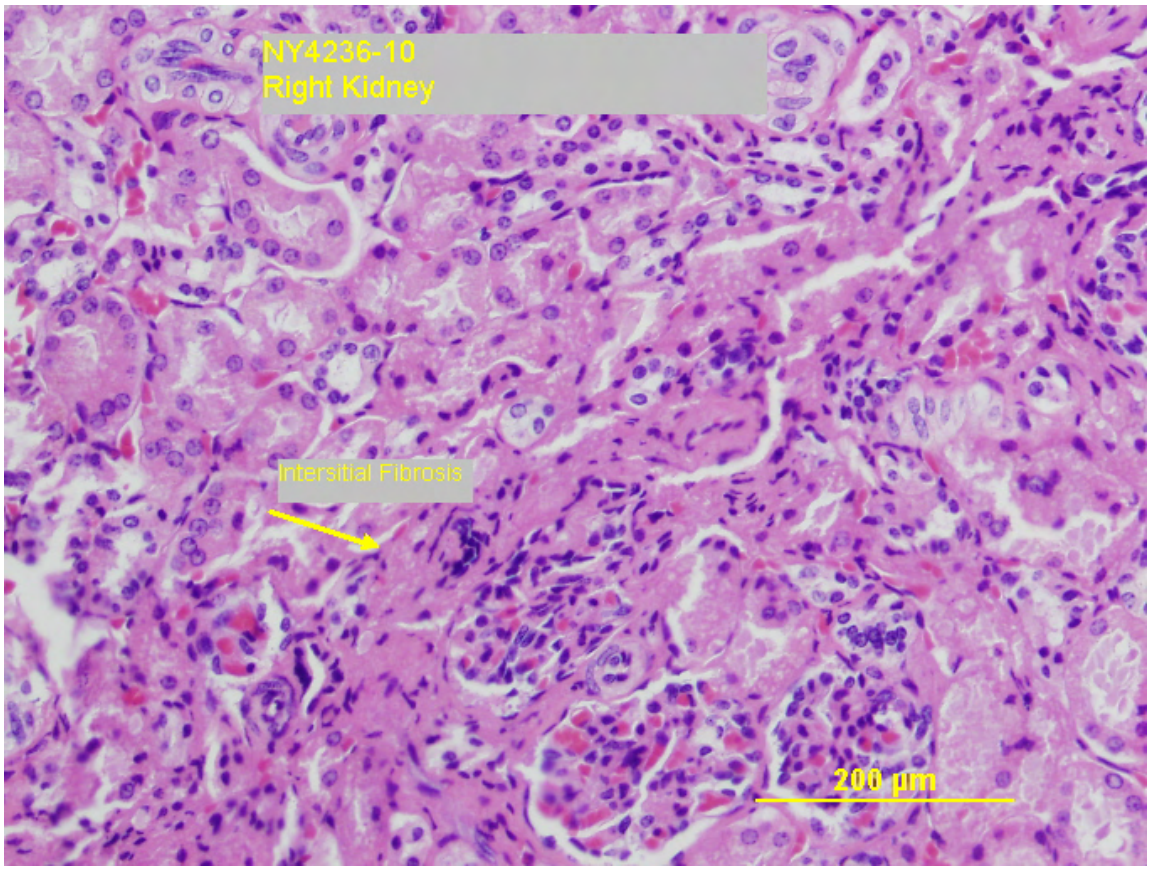



Figure 8.Kidney. There is loss of proximal tubules with replacement by fibrous connective tissue.

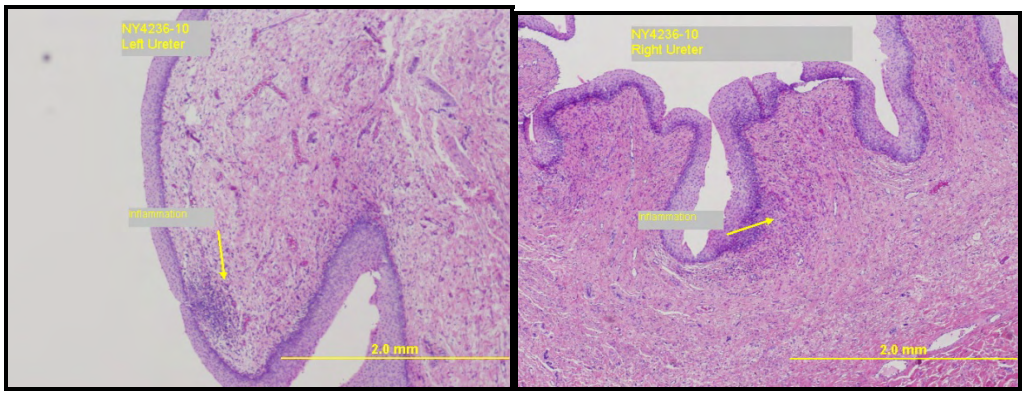



Figure 9a and 9b. Left and Right Ureter. The left ureteral submucosa is expanded by clear spaces (edema). Compare to the right ureter.

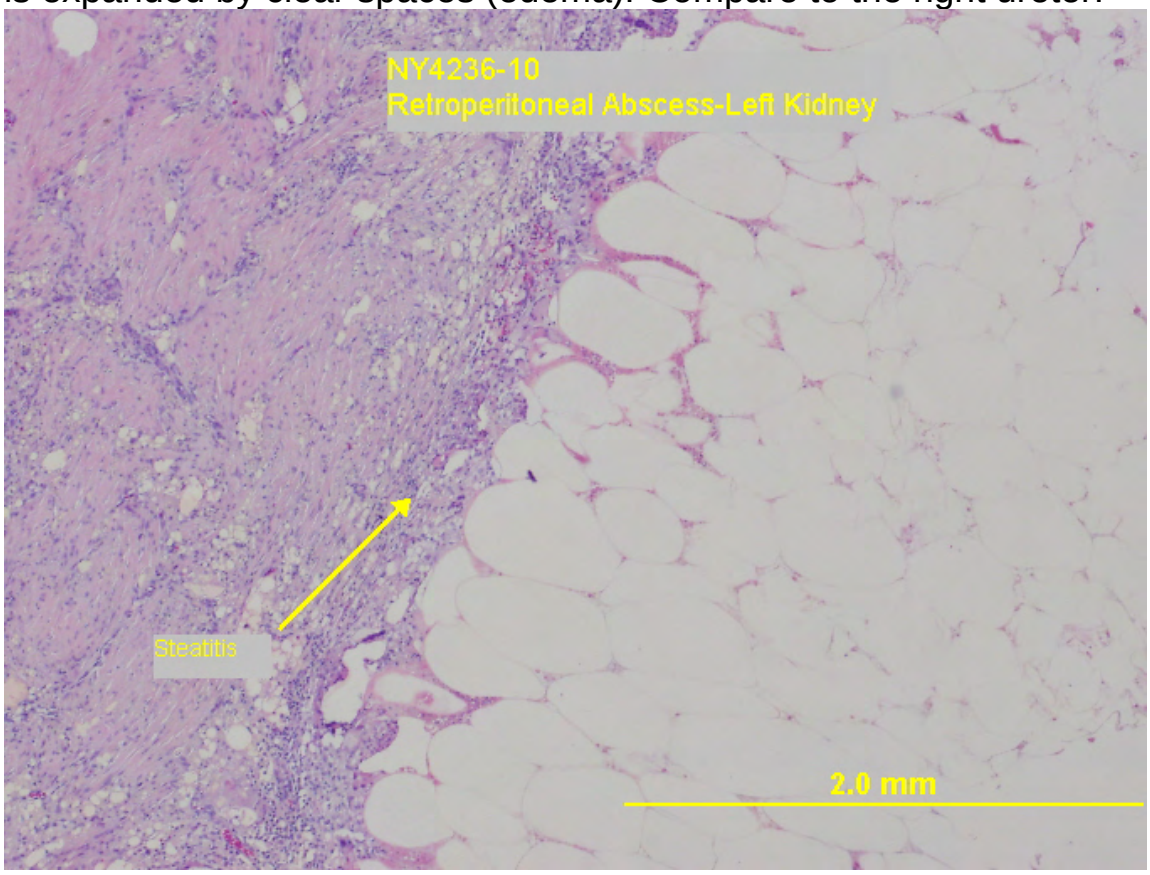



Figure 10. Retroperitoneal Abscess, Left Kidney. There is a thick fibrous capsule extending into the adipose.

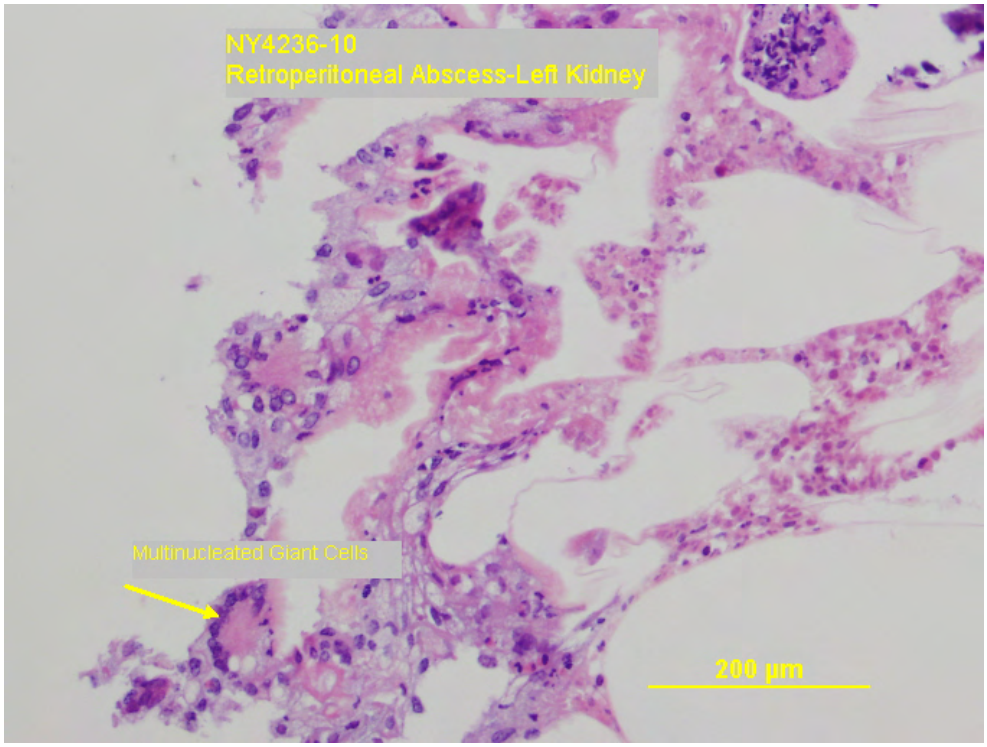



Figure 11. Retroperitoneal Abscess, Left Kidney. Multinucleated giant cells are present within the adipose.

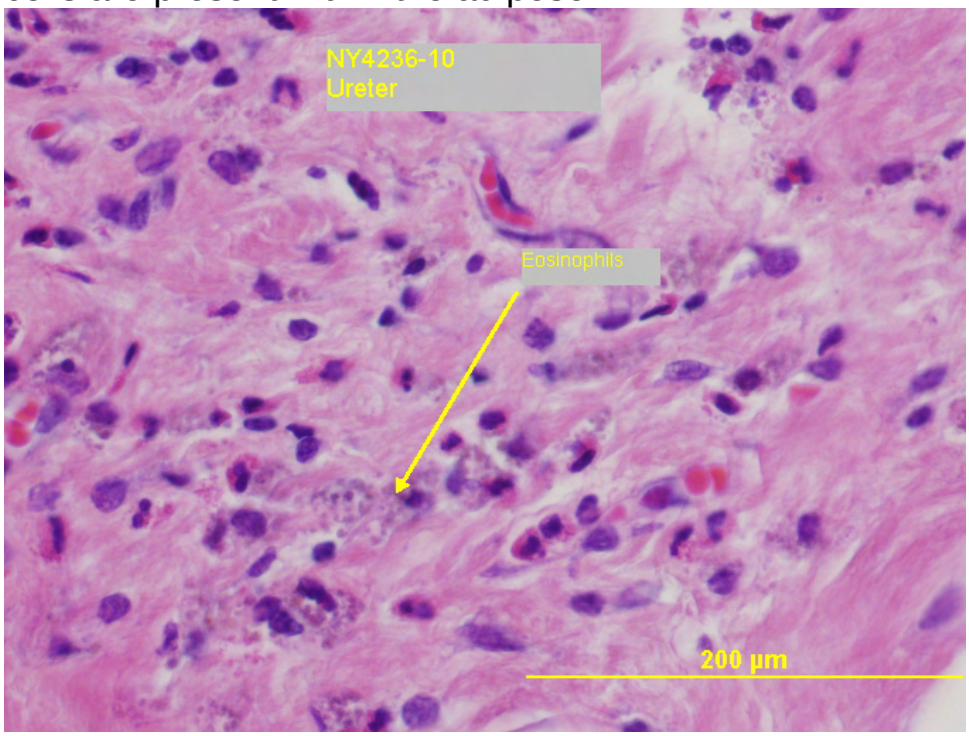



Figure 12. There are eosinophils and other inflammatory cells within a collagenous stroma. There is deposition of basophilic, granular substance (mineral).

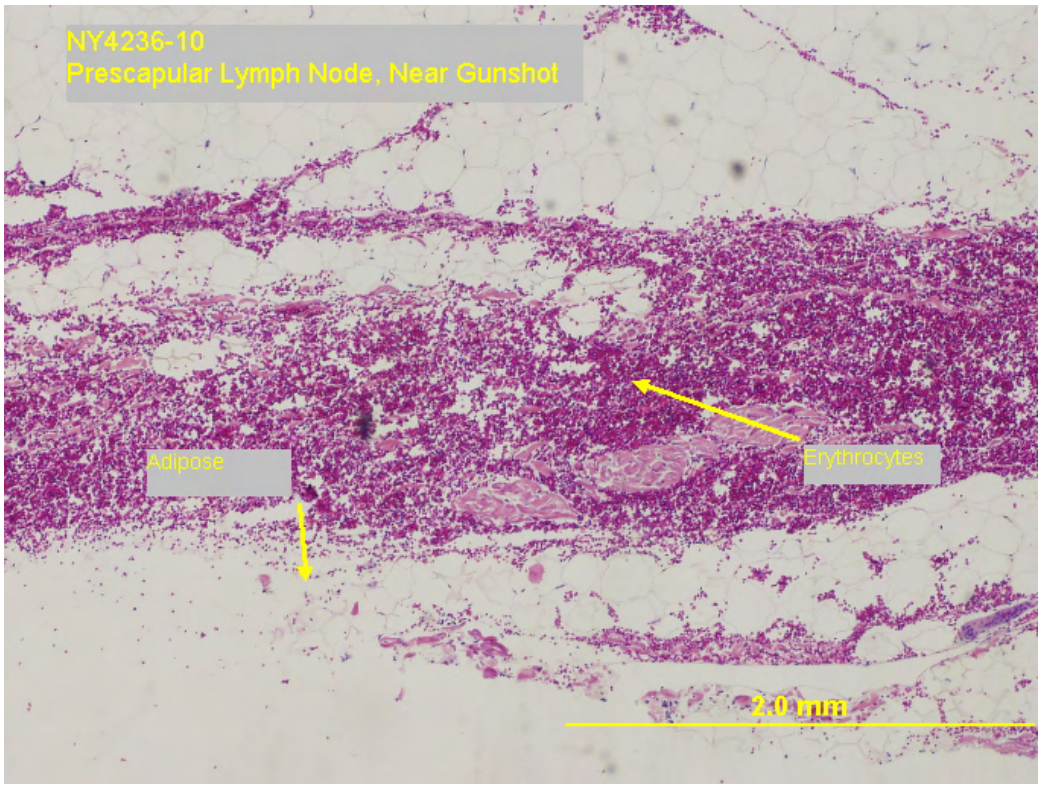



Figure 13.Prescapular Lymph Node. Within the perinodal adipose, there are bands of extravasated erythrocytes.

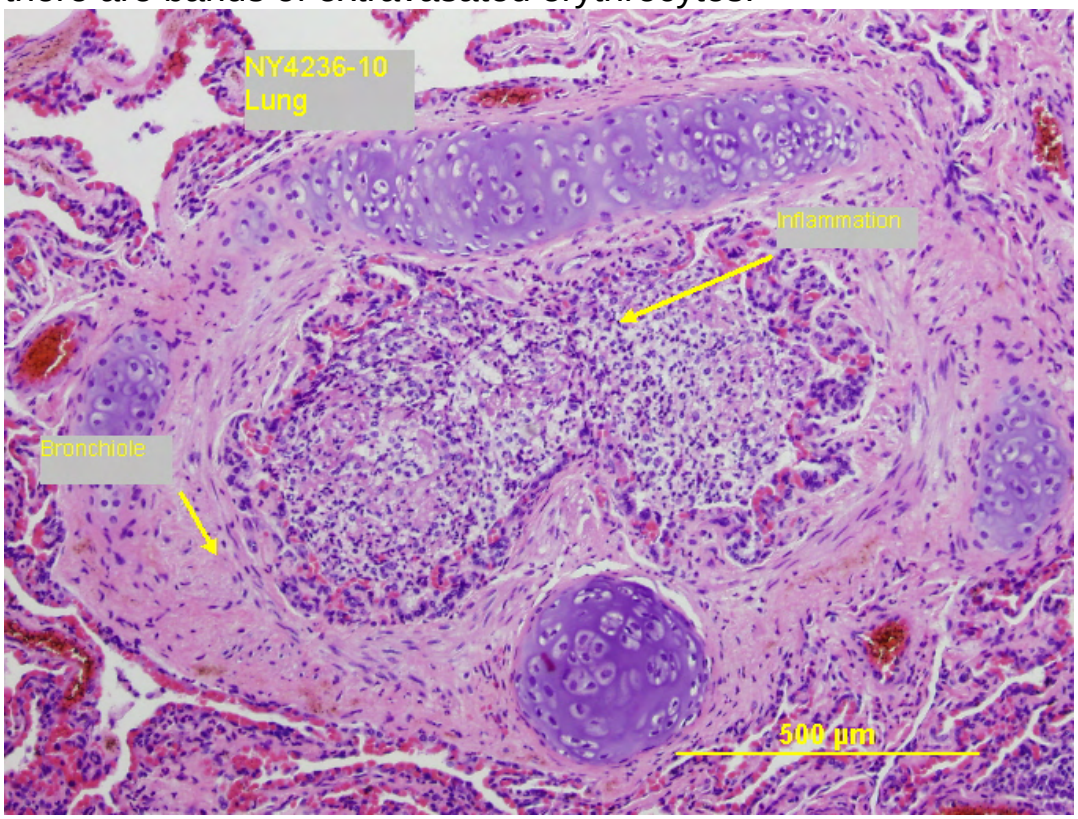



Figure 14.Lung. Bronchioles are filled with inflammatory cells.

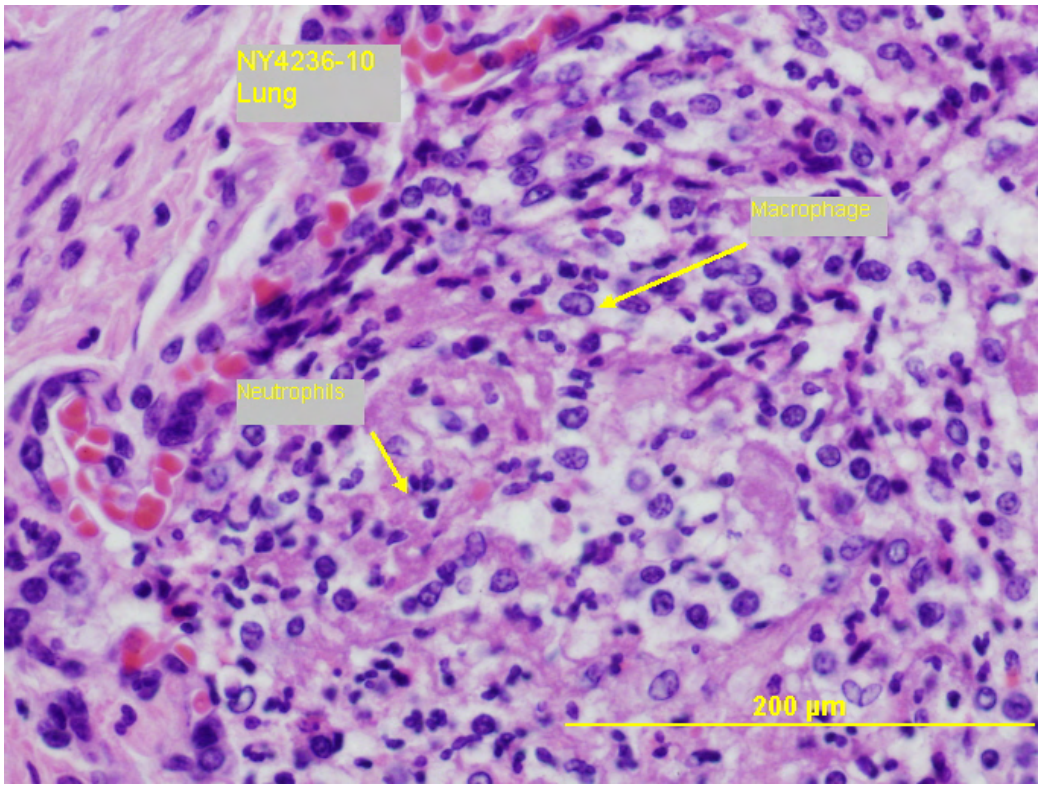



Figure 15.Lung, Bronchiole. Within the bronchiolar lumen, there are viable and degenerate neutrophils, macrophages, and fibrin.

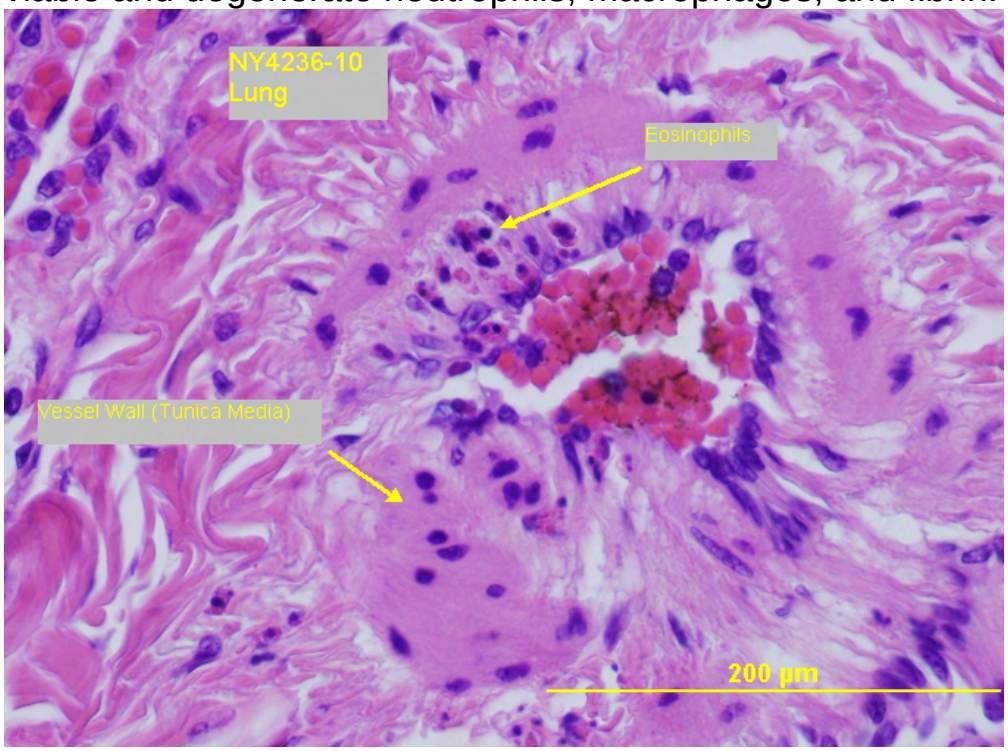



Figure 16.Lung. Eosinophils are sprinkled within the wall of the vessel.

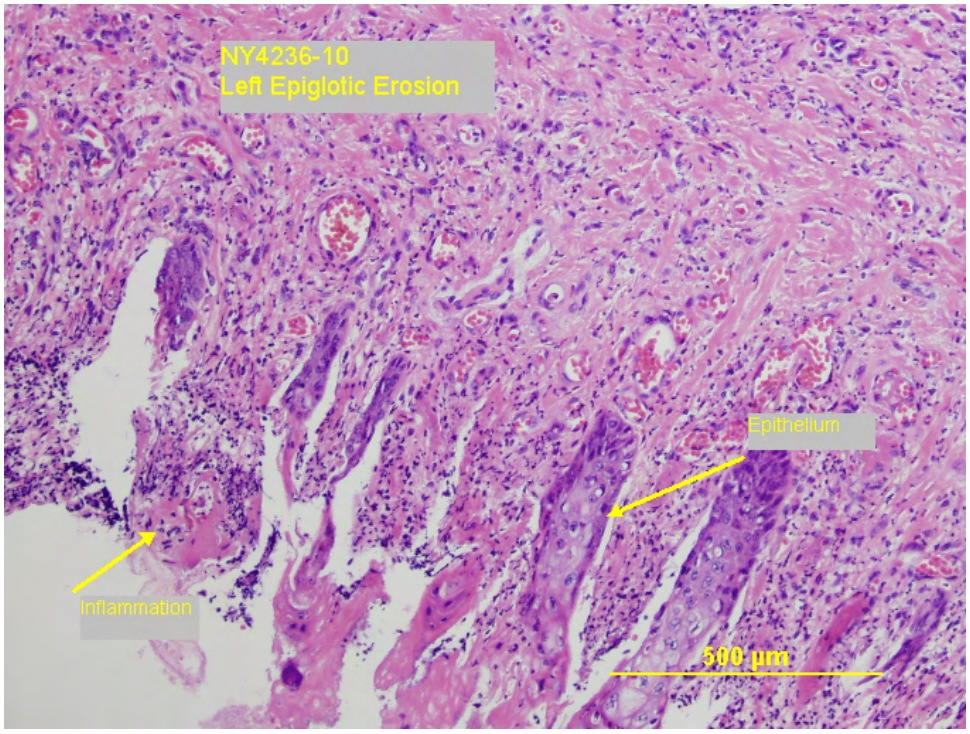



Figure 17. Left Epiglottis. There is ulceration of the epithelium with the defect covered by fibrin and neutrophils. Bacterial colonies are present within the necrotic foci (densely basophilic structure, lower left).

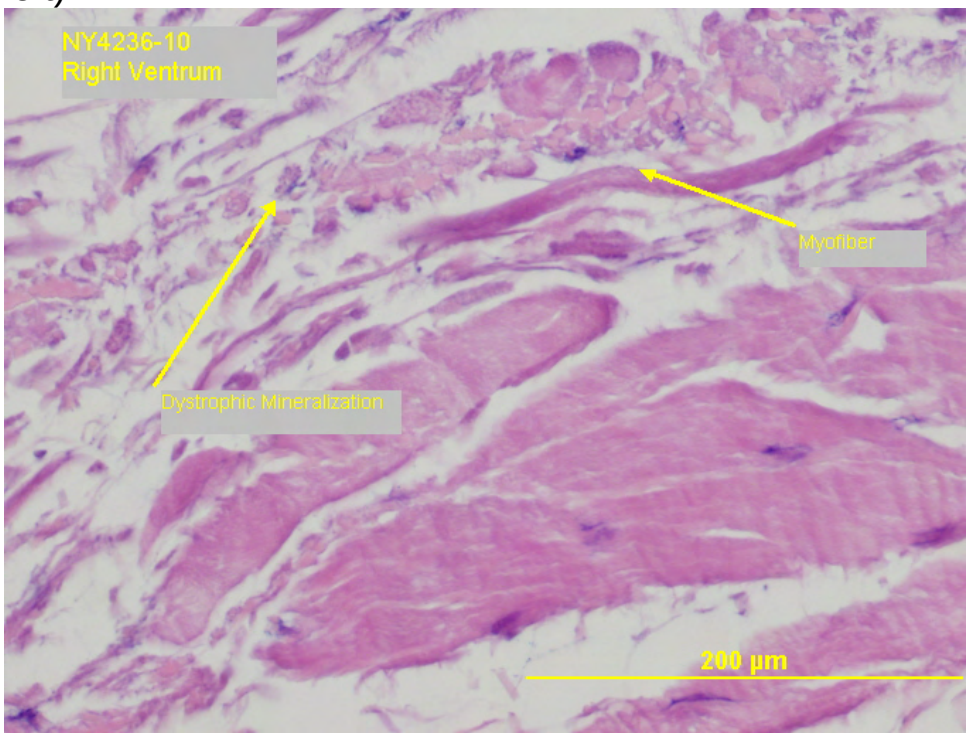



Figure 18. Collagen bundles are fragment, granular, and flanked by basophilic substance (dystrophic mineralization).

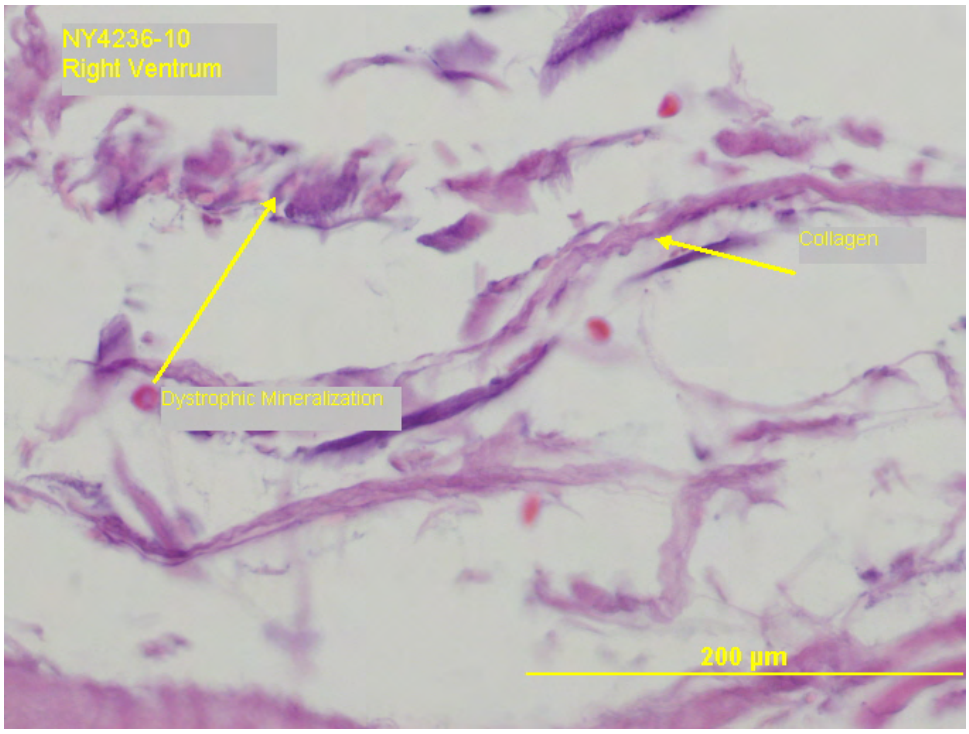



Figure 19. Ventrum. There are mineralized collagen fibers.

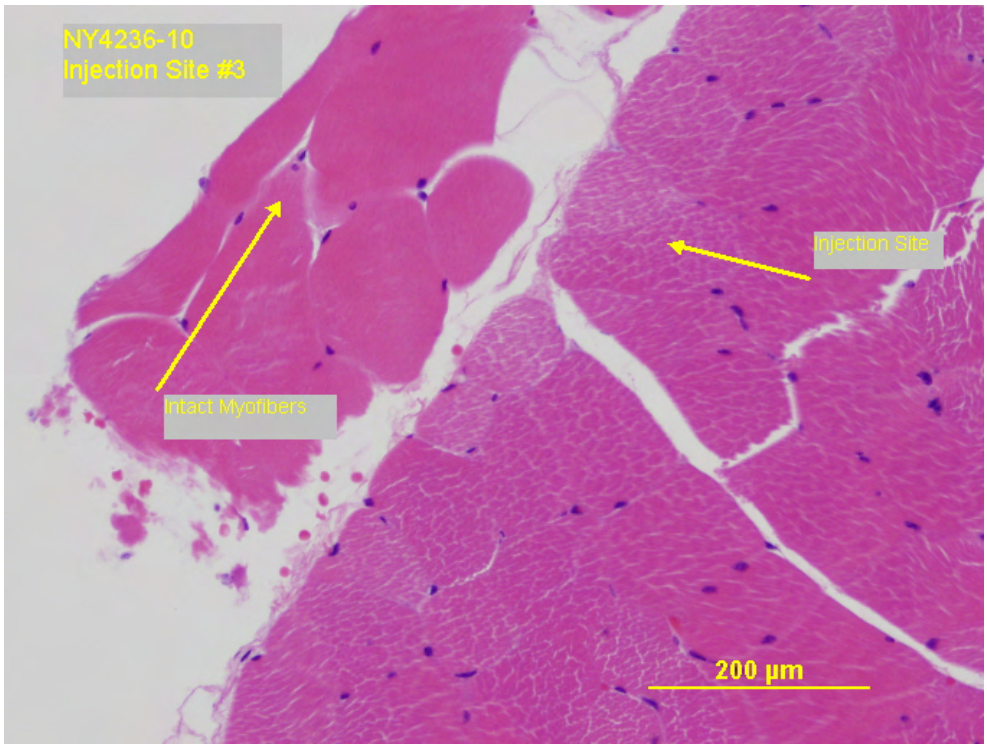



Figure 20. Injection Site 3. Adjacent to well-defined myofiber, there are myofibers that have poorly defined borders and are granular.

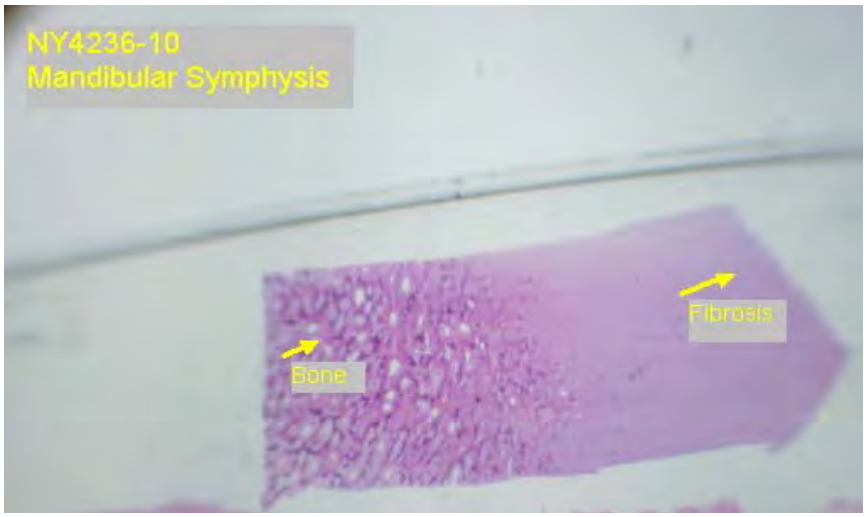



Figure 21.Mandibular Symphysis. There is a transition from woven bone to dense sheets of fibroblasts. (The “fuzziness” at the photo edge are a product of a subgross (12.5X) photomicrograph.

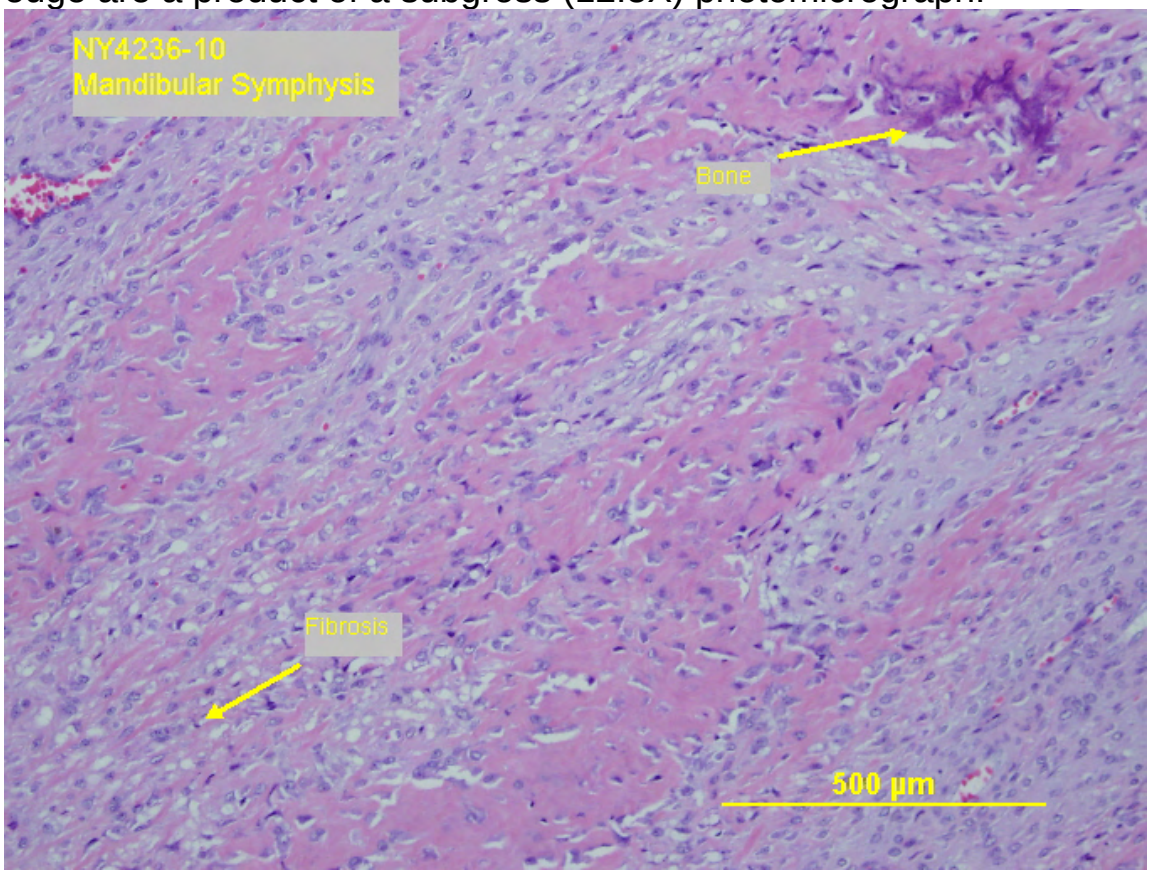



Figure 22. Mandibular Symphysis. Fibrocytes producing abundant collagenous matrix are present.

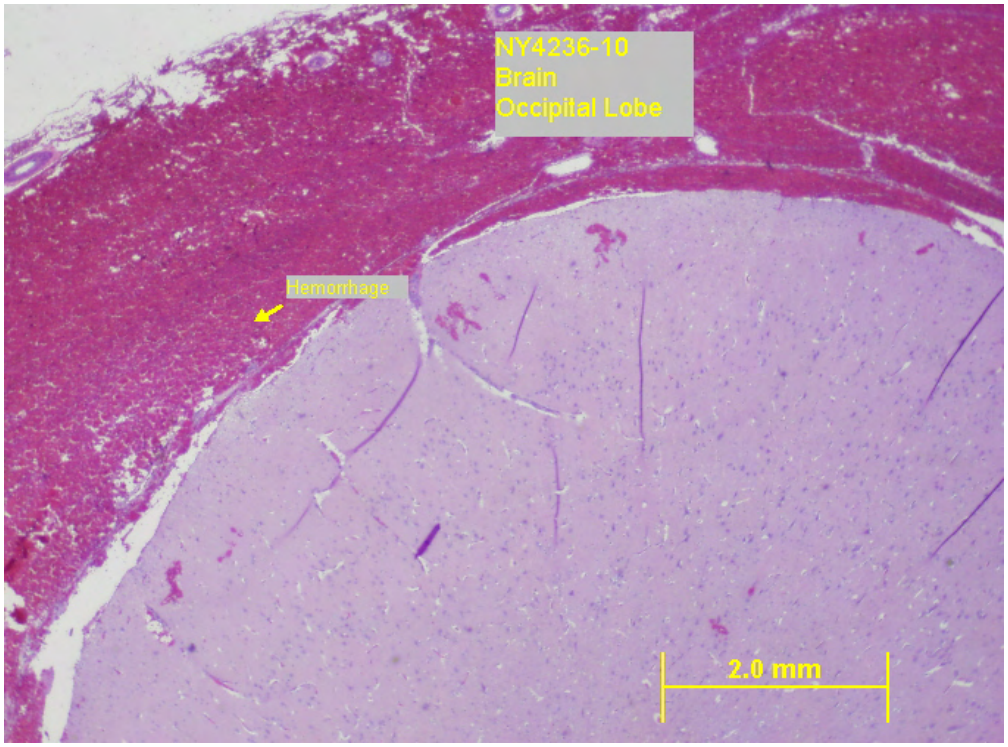



Figure 23. Occipital Lobe. There is hemorrhage within the subdural space.

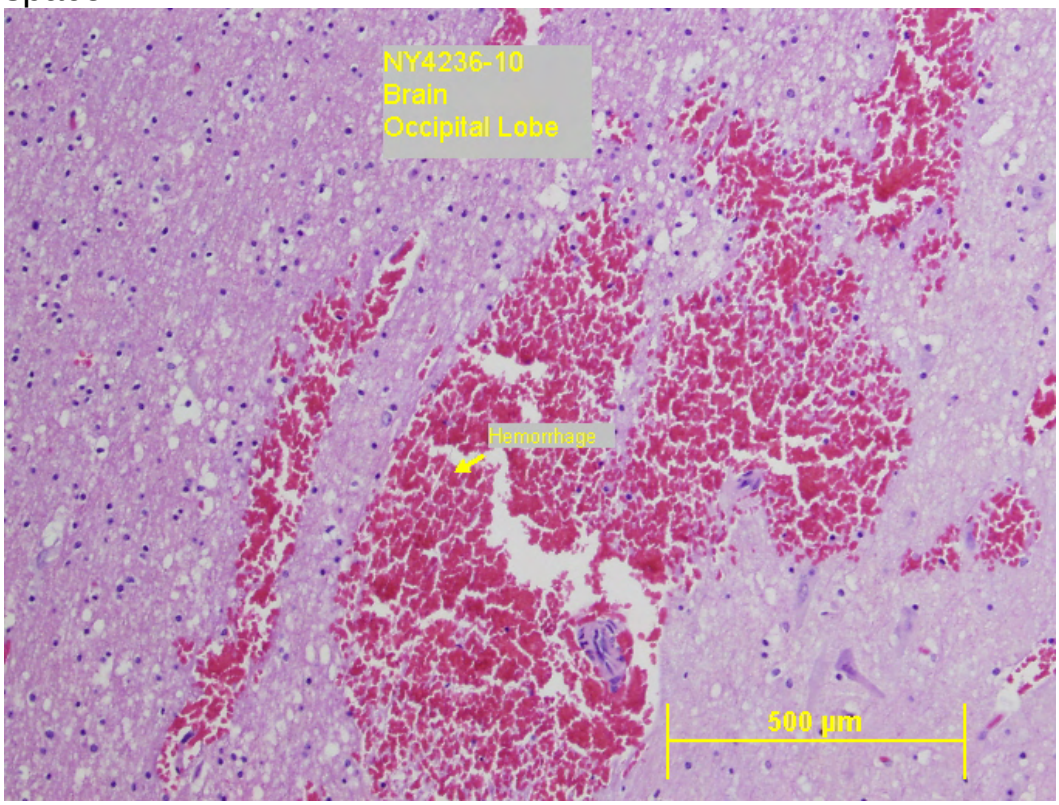



Figure 24. Occipital Lobe. There is hemorrhage within the neuropil.

| PENDING TESTS-PATHOLOGIST | | | |
|---|---|---|---|
| TEST | PURPOSE | SITE | RESULT |
| Histopath-brain | Microscopic examination | Brain | 6/15/2010 |
| | | | |
| | | | |

| DATE: | REPORTING PATHOLOGIST: |
|---|---|
| 07May2010 (preliminary) | David S. Rotstein, DVM, MPVM, DACVP |
| 18June2010 (final) | Denise McAloose, VMD, DACVP |

# APPENDIX 3 -

# LABORATORY ANALYSES

# Marine Mammal Stranding Report - Level A Data



**FIELD #:** NY4236-2010 **NMFS REGIONAL #:** NER10-1091 **NATIONAL DATABASE #:** NE-2010-1109467

**COMMON NAME:** HUMPBACK WHALE **GENUS:** Megaptera **SPECIES:** novaeangliae

**EXAMINER Name:** Kimberly Durham **Affiliation:** Riverhead Foundation for Marine Research and Preservation

**Address:** 467 E. Main St. Riverhead, NY 11901 US **Phone:** 631-369-9840

**Stranding Agreement or Authority:** Riverhead Foundation for Marine Research and Preservation

## LOCATION

**Country:** United States

**State:** NY **County:** Suffolk

**City:** East Hampton

**Body of Water:** Atlantic Ocean

**Locality Details:**

Approx. 800 yds East of Main Beach off of Ocean Avenue. East of 1st Jetty

**Lat:** 40.9454 **N**
decimal degrees actual

**Long:** -72.1870 **W**
decimal degrees actual

**How lat/long determined:** GPS

## OCCURRENCE DETAILS

[N] Restrand GE#:

**Group Event:** [N]

If yes, Type: [N] Cow/Calf Pair [N] Mass Stranding [N] UME # Animals: 1 actual

**Findings of Human Interaction:** N

[N] 1.Boat Collision [N] 2.Shot [N] 3.Fishery Interaction [N] 4.Other Human Interation:

**How Determined:** [N] 1. External Exam [N] 2. Internal Exam [Y] 3. Necropsy [N] 4. Other

**Gear Collected?** [N] **Gear Disposition:**

**Other Findings upon Level A:** N

[N] Illness [N] Injury [N] Pregnant [N] Other Findings

**Describe How Determined**

[N] External Exam [N] Internal Exam [Y] Necropsy

[N] Other

## INITIAL OBSERVATION

**Year:** 2010 **Month:** APR **Day** 06

**First Observed:** Beach or Land

**CONDITION AT INITIAL OBSERVATION:**

ALIVE

## LEVEL A EXAMINATION

[N] *Not Able to Examine*

**Year:** 2010 **Month:** APR **Day**: 09

**CONDITION AT EXAMINATION**

FRESH DEAD

## INITIAL LIVE ANIMAL DISPOSITION

[N] 1.Left at Site
[N] 2.Immediate Release at Site
[N] 3.Relocated
[N] 4.Disentangled
[N] 5.Died at Site
[Y] 6.Euthanized at Site
[N] 7.Transferred to Rehabilitation
[N] 8.Died During Transport
[N] 9.Euthanized During Transport
[N] 10. Other

### CONDITION/DETERMINATION

[N] 1.Sick
[N] 2. Injured
[N] 3. Out of Habitat
[N] 8. Unknown /CBD
[N] 4. Deemed Releasable
[N] 5. Abandoned /Orphaned
[N] 6. Inaccessible
[Y] 9. Other

7. Location Hazardous:
[N] a. To animal
[N] b. To public

**Comment:** Robust Body Condition. Determination for cause of stranding pending

## MORPHOLOGICAL DATA

**Sex** MALE

**Age Class** YEARLING

**Whole Carcass** [Y] **Partial Carcass** [N]

**Straight length:** 939.8 cm actual

**Weight:** 13000 kg estimate

**Photos/Videos taken:** Y

**Disposition:**

RFMRP Digital Image Archive

## TAG DATA

Tags Were:
Present at Time of Stranding (pre-existing): N
Applied during Stranding Response: N

| ID# | Color | Type | *Placement | Applied | Present |
|---|---|---|---|---|---|
| | | | | N | N |
| | | | | N | N |
| | | | | N | N |
| | | | | N | N |

* D=Dorsal; DF=Doral Fin; L-Lateral Body
LF=Left Frontl LR=Left Rear; RF=Right Front; RR=Right Rear;

## WHOLE CARCASS DISPOSAL (Check One or more)

[N] 1.Left at Site
[N] 2.Buried
[N] 3.Rendered
[Y] 7.Landfill
[N] 9.Other:
[N] 4.Towed Lat Lon
[N] 5.Sunk Lat Lon
[N] 6.Frozen for Later Examination
[N] 8.Unknown

**SPECIMEN DISPOSITION**

[Y] 1.Scientific Collection [N] 2.Educational Collection
[N] 3.Other:

**Comments:**

**NECROPSIED** Y complete fresh

**NECROPSIED BY:** William McLellan

**Date:** 2010-APR-09

# Marine Mammal Stranding Report - Level A Data



## ADDITIONAL REMARKS

**Additional Identifier:**

**Additional Remark:**

### DISCLAIMER

These data should not be used out of context or without verification. This should be strictly enforced when reporting signs of human interaction data.

### DATA ACCESS FOR LEVEL A DATA

Upon written request, certain fields of the Level A Data Sheet will be released to the requestor provided that the requestor credit the stranding network and the National Marine Fisheries Service (NOAA Fisheries). The National Marine Fisheries Service will notify the contributing stranding network members that these data have been requested and the intent of use. All other data will be released to the requestor provided that the requestor obtains permission from the contributing stranding network and the National Marine Fisheries Service.

### PAPERWORK REDUCTION ACT INFORMATION

Public reporting burden for the collection of information is estimated to average 20 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of the collection of information, including suggestion for reducing the burden, to: Chief, Marine Mammal Conservation Division, Office of Protected Resources, National Marine Fisheries Service (NOAA Fisheries), 1315 East-West Highway, Silver Spring, Maryland 20910. Notwithstanding any other provision of the law, no person is required to respond to, nor shall any person be subjected to a penalty for failure to comply with, a collection of information subject to the requirements of the Paperwork Reduction Act, unless the collection of information display a currently valid Office of Management and Budget (OMB) Control Number.

**NEW YORK STATE**
**MARINE MAMMAL AND SEA TURTLE STRANDING NETWORK**

CETACEAN DATA RECORD

Field # NY4236-2010

Species: Megaptera novaeangliae
Date of Stranding/Capture: 04 / 06 /2010
Number of Individuals: 1
Time of Stranding/Recover: 10:30
Sex: (M) / F / U Observer(s): RF,UNC Date/Time of Post Mortem: 4/9/2010 /13:00 am/pm Performed By: W. McLellan
Reported By: East Hampton Town Police
Phone Number: 631-324-0777
Location: Nearest Street Main Beach East Hampton
City/Village East Hampton
County Suffolk
Beach # 144
Lat/ Long (degrees) 40 56'43.4" N / 72 11'13.2" W
(decimals) 40.9454 N / -72.1870 W
Nearest Body Of Water Atlantic Ocean
Condition of Specimen(s): Alive - Died following euthanasia 6/9/2010

Circumstances of Stranding: Multi-organizational response with NOAA as IC. Remote sedation followed by ballistics and chemical euthanasia

Tags/Marks/Scars:
Released: Date ____/____/____ Tag# _____ Tag Type _____
Photos Taken: NO (YES) Total # _____ Roll # _____ Photo # _____
Euthanized: NO (YES) Date 04 / 09 / 2010

Original measurements collected in inches and converted to cm. (4/9/10). Trans. by KD

**MEASUREMENTS**
PRIMARY

| | | |
|---|---|---|
| 1. Length, Total (tip of upper jaw to notch) | Straight: | 939.8 cm |
| | Curved: | n/a |
| 2. Length (tip of upper jaw to center of eye) | | 212.1 cm |
| 3. Length of gape (tip of upper jaw to angle of gape) | | 206.4 cm |
| 4. Length, tip of upper jaw to anterior insertion of flipper | | 284.8 cm |
| 5. Length, tip of upper jaw to tip of dorsal fin | | 632.5 cm |
| 6. Length, tip of upper jaw to apex of melon | | n/a |
| 7. Length, tip of upper jaw to external auditory meatus | | 261.0 cm |
| 8. Center of eye to external auditory meatus | | 45.7 cm |
| 9. Center of eye to angle of gape | | 22.9 cm |
| 10. Center of eye to center of blowhole(s) | | 119.4 cm |
| 11. Length, flipper (anterior insertion to tip) | | 280.7 cm |
| 12. Length, flipper (axilla to tip) | | 260.4 cm |
| 13. Width, flipper (maximum) | | 83.8 cm |
| 14. Width, flukes (tip to tip) | | 325.1 cm |
| 15. Depth of notch ( if none, so state) | | n/a |
| 16. Length, tip of upper jaw to center of anus | | 658.0 cm |
| 17. Length, tip of upper jaw to midpoint of umbilicus | | 538.0 cm |
| 18. Length of upper jaw to midpoint of genital aperture | | 622.0 cm |
| 19. Length, mammary slits: | Right: | n/a |
| | Left : | n/a |
| 20. Length, genital slit: | | n/a |
| | Anal: | n/a |
| 21. Girth: maximum | | n/a |
| 22. Girth: on transverse plane intersecting axilla | | n/a |
| Mouth to Blowhole | | 172.7 cm |
| Fluke Notch to Anus | | 300 cm |

Field # NY 4236-2010

23. Girth on transverse plant intersecting anus n/a
24. Blubber thickness, mid dorsal ant. Insertion of dorsal fin n/a
25. Blubber thickness, mid-lateral at mid length n/a
26. Blubber thickness, mid-ventral at mid length n/a
27. Length, throat creases: Maximum: n/a
Minumum: n/a
28. Weight 13000 estimate kg

Blubber Thickness: Mid lateral 7.3 cm
Blubber Thickness: Blowhole 8.1 cm
Blubber Thickness: 1 lat atsame (?) 9.3 cm

## GROSS NECROPSY INFORMATION

EXTERNAL

**GENERAL CONDITION**

Nutritional State: _____

Epidermis: _____

Oral: _____ Eyes: _____

Nasal: _____ Ears: _____

Penile/Vulvar: _____ Anal: _____

Peripheral Lymph nodes: _____

Palpable Abnormalities/Physical Discrepancies: _____

_____

_____

_____

External Parasites: _____

_____

_____

Number Of Teeth:

Right Upper: _____ Left Upper: _____

Right Lower: _____ Left Lower: _____

INTERNAL

**ABDOMINAL CAVITY**

Adrenal _____

Bile Ducts _____

Bladder _____

Duodenum _____

Intestines Small _____

Large _____

Kidney Right _____

Left _____

Liver _____

Mammary Glands _____

Mesenteric Lymph Nodes _____

Ovaries _____

Pancreas _____

Peritoneum _____

Spleen _____

Stomach _____

Testes _____

Ureters _____

Urethra _____

Uterus/Fetus _____

**THORACIC CAVITY**

Diaphragm _____

Heart (including valves) _____

_____

Lungs _____

Mediastinal Lymph Nodes _____

Parathyroids _____

Pleura _____

Pulmonary Artery _____

Trachea and Mainstem Bronchi _____

Thymus _____

Thyroids _____

**SUPPLEMENTAL**

Bones _____

Bone Marrow _____

Brain _____

Ears _____

Eyes _____

Mucous Membranes _____

Muscles _____

Spinal Cord _____

Teeth _____ Number/Type Removed _____

Tongue _____

**Comments/Notes**

20 cm dorsal of entrance , large areas of torn, clotted musculature (from ballistics) Sample collected just dorsal from transverse process.

Field # NY 4236-2010

## TISSUES COLLECTED

| TISSUE COLLECTED | HIST | TOX | BACT | VIR | TISSUE COLLECTED | HIST | TOX | BACT | VIR |
|---|---|---|---|---|---|---|---|---|---|
| Adrenal | | | | | Stomach Fore | | | | |
| Bladder | | | | | Fundus | | | | |
| Bone Marrow | | | | | Antrum | | | | |
| Brain | | | | | Pylorus | | | | |
| Bronchi | | | | | Testes | | | | |
| Duodenum | | | | | Thyroids | | | | |
| Esophagus | | | | | Thymus | | | | |
| Fat | | | | | Trachea | | | | |
| Gall Bladder | | | | | Ureter | | | | |
| Heart | | | | | Uterus | | | | |
| Heart Valves | | | | | **OTHER TISSUES** | | | | |
| Ileum | | | | | | | | | |
| Intestine, Small | | | | | | | | | |
| Intestine, Large | | | | | | | | | |
| Jejunum | | | | | | | | | |
| Kidney | | | | | | | | | |
| Larynx | | | | | | | | | |
| Liver | | | | | | | | | |
| Lungs | | | | | | | | | |
| Mediastinal LN | | | | | | | | | |
| Mesenteric LN | | | | | | | | | |
| Ovaries | | | | | | | | | |
| Pancreas | | | | | | | | | |
| Parathyroid | | | | | | | | | |
| Peripheral LN | | | | | **SPECIAL REQUESTS** (Specify tissue type and name of facility sent to) | | | | |
| Peritoneum | | | | | | | | | |
| Prostate | | | | | | | | | |
| Salivary Glands | | | | | | | | | |
| Skin | | | | | | | | | |
| Spleen | | | | | | | | | |
| Spinal Chord | | | | | | | | | |

**Patient Info:**
**Name:** Mnovangliae
**Chart No:**
**Owner:** Ny4236 10
**Doctor:**
**Species:** Mammal
**Breed:**
**Age:**
**Sex:** U

**Hospital:**
Riverhead Found-Marine Res & Pres
467 E Main St
Riverhead, NY 11901
**Antech ID:** 7798

**Lab:**
Antech Diagnostics
1111 Marcus Avenue
Lake Success, NY 11042
**Reported:** 04/16/10 01:54 PM
**Received:** 04/10/10

| Accession No. | Doctor | Owner | Pet Name |
|---|---|---|---|
| **NYAA85517508** | | **Ny4236 10** | **Mnovangliae** |

| Test | Results | Adult Reference Range | L | Normal | H |
|---|---|---|---|---|---|
| **T4** | | | | | |
| T4 | 20.0 | µg/dL | | | |
| Verified by repeat analysis. | | | | | |
| **T3** | | | | | |
| T3 | 260 | ng/dL | | | |
| **Superchem** | | | | | |
| Total Protein | 7.0 | 7.0-8.0 g/dL | | | |
| Albumin | 4.5 | 3.5-4.5 g/DL | | | |
| Globulin | 2.5 | g/dL | | | |
| **Albumin/Globulin Ratio** | **1.8** | **0.9-1.0 RATIO** | **HIGH** | | |
| AST (SGOT) | 88 | U/L | | | |
| ALT (SGPT) | 7 | U/L | | | |
| Alkaline Phosphatase | 370 | U/L | | | |
| GGTP | 5 | U/L | | | |
| Total Bilirubin | 0.2 | mg/dL | | | |
| Urea Nitrogen | 52 | mg/dL | | | |
| Creatinine | 2.9 | mg/dL | | | |
| BUN/Creatinine Ratio | 18 | RATIO | | | |
| **Phosphorus** | **4.5** | **6.0-8.0 mg/dL** | **LOW** | | |
| Glucose | 169 | mg/dL | | | |
| **Calcium** | **10.5** | **8.0-9.0 mg/dL** | **HIGH** | | |
| Magnesium | 2.7 | mEq/L | | | |
| Sodium | 150 | 149-158 mEq/L | | | |
| **Potassium** | **3.1** | **3.8-4.2 mEq/L** | **LOW** | | |
| Na/K Ratio | 48 | | | | |
| Chloride | 111 | 108-119 mEq/L | | | |
| Cholesterol | 553 | mg/dL | | | |
| Triglycerides | 131 | mg/dL | | | |
| Amylase | 13 | U/L | | | |
| Lipase | 10 | U/L | | | |

| Accession No. | Doctor | Owner | Pet Name |
|---|---|---|---|
| NYAA85517508 | | Ny4236 10 | Mnovangliae |

| Test | Results | Adult Reference Range | L | Normal | H |
|---|---|---|---|---|---|
| CPK | 2517 | U/L | | | |
| **CBC** | | | | | |
| **Hemoglobin** | **9.4** | **15.0-17.0 g/dL** | **LOW** | | |
| **Hematocrit** | **27.8** | **43.0-49.0 %** | **LOW** | | |
| **WBC** | **2.2** | **6.0-9.0 $10^3$/µL** | **LOW** | | |
| Verified by repeat analysis. | | | | | |
| **RBC** | **2.01** | **3.00-5.00 $10^6$/µL** | **LOW** | | |
| MCV | 138 | fl | | | |
| MCH | 46.8 | pg | | | |
| MCHC | 33.8 | g/dl | | | |
| Platelet Count | 23 | $10^3$/µL | | | |
| Specimen contained fibrin clots which may invalidate results. | | | | | |
| Platelet Estimate | Decreased | | | | |
| **Differential** | **Absolute** | **%** | | | |
| Neutrophils | 1320 | 60 | | | |
| Bands | 0 | 0 | | | |
| Lymphocytes | 484 | 22 | | | |
| Monocytes | 110 | 5 | | | |
| Eosinophils | 286 | 13 | | | |
| Basophils | 0 | 0 | | | |
| Polychromasia | None | | | | |
| Blood Parasites | None Seen | | | | |
| **Blood Culture** | | | | | |
| Source: | Blood | | | | |
| Preliminary #1 | 04/12/2010 | | | | |
| No growth after 24 hours. | | | | | |
| Preliminary #2 | 04/13/2010 | | | | |
| Bacteria isolated. Identification and sensitivity to follow. | | | | | |
| Preliminary #3 | 04/15/2010 | | | | |
| Organism #1 | Staph Species | | | | |
| Staph Speciation | | | | | |
| Staphylococcus epidermidis | | | | | |

| Sensitivities | #1 | #2 | #3 | #4 | #5 |
|---|---|---|---|---|---|
| Ampicillin | R | | | | |
| Clavamox | S | | | | |
| Chloramphenicol | S | | | | |

| Accession No. | Doctor | Owner | Pet Name |
|---|---|---|---|
| **NYAA85517508** | | **Ny4236 10** | **Mnovangliae** |

| Test | Results | Adult Reference Range | L | Normal | H |
|---|---|---|---|---|---|
| Clindamycin | S | | | | |
| Cephalothin | S | | | | |
| Methacillin | S | | | | |
| Erythromycin | R | | | | |
| Enrofloxacin | S | | | | |
| Gentamicin | S | | | | |
| Neomycin | S | | | | |
| Trimethoprim/Sulfa | S | | | | |
| Tetracycline | S | | | | |
| Marbofloxacin | S | | | | |
| **Vitamine A** | | | | | |
| Vitamin A | | | | | |

50.0 ng/mL
NO REFERENCE RANGE AVAILABLE FOR THIS SPECIES.
TEST PERFORMED AT ANIMAL HEALTH DIAGNOSTIC LABORATORY AT MSU

| Testosterone | | | | | |
|---|---|---|---|---|---|
| Testosterone | <40.0 | pg/ml | | | |

**Accession#: C101110471**

# Texas Veterinary Medical Diagnostic Laboratory System

## Final Report

**P.O. Drawer 3040, College Station, TX 77841-3040**
**Phone: (888)646-5623 Fax: (979)845-1794 http://tvmdl.tamu.edu/**

**(718)220-7103**

**Owner's Name:**
NY4236-10, Humpback Whale

**Veterinarian/Submitter: Account ID#: 11567**
Wildlife Conservation Society
Attention: Dr. Paul Calle
185th Street & Southern Boulevard
Bronx, NY 10460

**Date specimens received:** 4/21/2010

**Preliminary reports:**
**Phone reports:**
**Final report:** Email 4/21/2010

**Species:** Exotic **Age:** **Animal ID:** NY4236-10
**Breed:** **Weight:** 13000 Pounds **Sex:** Male

**Tests Requested:** Urine Myoglobin

**Specimens Submitted:** Urine Sample

**Clinical History:** 4/20/10 - Stranded 2-3 year old humpback whale. Animal was euthanized. Sample submitted is post mortem. /kg

**Clinical Diagnosis:**

**Previous Cases:**

**Treatment:**

**Conclusion/Interpretation of Lab Findings**:
Laboratory results as listed. J. A. Akins, DVM/bw

**Accession#: C101110471**

# Texas Veterinary Medical Diagnostic Laboratory System

## Final Report

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**Clinical Diagnosis:**

**Previous Cases:**

**Treatment:**

**Conclusion/Interpretation of Lab Findings**:
Laboratory results as listed. J. A. Akins, DVM/bw

**Accession#: C101110471**

| *LABORATORY TEST STATUS:* | **Ordered** | **Current Status** |
|---|---|---|
| Urine myoglobin/hemoglobin (C) | 4/21/2010 | Completed 4/21/2010 |

## Clinical Pathology

**Completed:** 4/21/2010

**Test:** Urine myoglobin/hemoglobin **Specimen:** Urine

| **Animal ID** | **Hemoglobin** | **Myoglobin** |
|---|---|---|
| NY4236-10 | Negative | Negative |

**Comment:**
--- J. Akins, DVM,MS
/bw

## Veterinary Bulletin:

**Vitamin A Testing Temorarily Forwarded:**
Due to an instrumentation maintenance issue, the TVMDL Toxicology Section will not be able to provide testing for Vitamin A for approximately the next 3 weeks. You may continue to send samples for Vitamin A testing during this time period, however, they will be forwarded to another Laboratory, until our equipment is functional again. Results for those tests will be provided on our normal TVMDL report and a nominal specimen forwarding fee may apply.

***UPDATED*** **: Trichomonas testing by TAHC Regulation effective January 1, 2010:**

By TAHC Regulation, testing for Trichomoniasis is now a "Program Disease" requiring documentation of the test results and reporting of both positive and negative results to TAHC. TVMDL is directed to provide "official test results" for specimens submitted on the TAHC "Trich Test Record" which is available on line at http://www.tahc.state.tx.us/animal_health/trich/09-03_TrichomoniasisTestRecord.pdf
Tests request for an "Official" Trich test by either culture or PCR must be accompanied by the TAHC Trich Test Record. The "official test" result is required in a change of possession, crossing state line, etc. for a bull of breeding age. A TVMDL Submission form is also requested to be submitted with the test request. When testing is completed, TVMDL will return the Test Record TO THE VETERINARIAN BY US MAIL for distribution as required by regulation. VETERINARINS WITH EMAIL REPORTING OF THEIR TVMDL RESULTS CAN RECEIVE AN EMAIL OF THE TVMDL CASE REPORT IMMEDIATELY WHEN THE CASE IS FINAL.
A veterinarian may also request an "Un-Official Test" or a "Diagnostic" Trich test which is ALSO done by USING THE TAHC TRICH TEST RECORD SUBMISSION FORM. By TAHC Regulation, results from diagnostic tests are also reportable to TAHC (electronic notification by TVMDL). POSITIVE RESULTS ON A DIAGNOSTIC TEST WILL INITIATE REGULATORY ACTION AS REQUIRED BY TAHC REGULATION.
TVMDL does not have the authority to change the status of a Trich test. Therefore, if you have submitted a case for Trich testing on a TVMDL Submission form and wish to change the status of that "diagnostic test" to an "official test," you must coordinate that request through your local TAHC Area Office or call the Austin Central Office at 512-719-0700 AFTER YOU RECEIVE YOUR TVMDL TEST RESULTS.

**COMPLETION OF THE VS 10-11 EIA FEDERAL SUBMISSION FORM**
As a Federal program disease, Equine Infectious Anemia testing can only be provided in Texas by submitting a properly completed Federal Form VS 10-11 with the specimen to be tested. As instructed by Veterinary Services Memorandum No. 555.16, TVMDL is prohibiting from testing samples submitted for EIA testing if this form is NOT PRESENT OR NOT PROPERLY COMPLETED by an

**Accession#: C101110471**

accredited veterinarian. VS Forms 10-11 that are not complete must be sent by TVMDL to the USDA office in Austin, Texas or to the Texas Animal Health Commission for follow up. Please insure you have properly completed this form to prevent delays in releasing results to your clients.

---

**Changes in Johne's Disease ELISA Values:**

The test used to serologically test for Johne's Disease will soon be modified and new "S/P" cut off values will be reported. Beginning May 1, 2010 the new S/P cut off value will be reported as >0.60 rather than the older value of >0.25. Results will be released as Negative = <0.60, Suspicious = > 0.60 and < 0.70, and Positive > 0.70. This change will not alter the utilization of tests results, but it will involve some interpretation, if the new S/P values are compared to older tests results with a cut off of >0.25.

Cornell University
Animal Health Diagnostic Center

Upper Tower Road, Ithaca, NY 14853
Ph: 607-253-3900 Fax: 607-253-3943
http://diagcenter.vet.cornell.edu

## *Finalized Report*

Accession Number: **39369-10**

Owner: Wcs Department Of Pathology

Premise ID: 00BZKEP

Wildlife Health Center - (784)
Bronx Zoo
2300 Southern Blvd
Bronx, NY 10460
(718) 220-7105

Sampled: 04/09/2010
Received: 04/13/2010
Finalized: 04/16/2010
Reference Number: NY4236 10

# BACTERIOLOGY

607-253-3900

### Aerobic Bacterial Culture

| Item | | Result |
|---|---|---|
| 1 | NY4236 10 HUMPBACK WHALE - Cetacea Whale, Nos Male Jejunum | Many Listonella Anguillarum |

### Anaerobic Bacterial Culture

| Item | | Result |
|---|---|---|
| 1 | NY4236 10 HUMPBACK WHALE - Cetacea Whale, Nos Male Jejunum | No Growth |

### Campylobacter jejuni Culture

| Item | | Result |
|---|---|---|
| 1 | NY4236 10 HUMPBACK WHALE - Cetacea Whale, Nos Male Jejunum | No Campylobacter Jejuni |

### Salmonella Culture

| Item | | Result |
|---|---|---|
| 1 | NY4236 10 HUMPBACK WHALE - Cetacea Whale, Nos Male Jejunum | No Salmonella Sp Isolated |

National Marine Life Center
120 Main Street, PO Box 269
Buzzards Bay - Cape Cod, MA
02532-0269
rwilliams@nmlc.org

# Parasitology Report

case# NMLC P 10-17

**Submitted by:** Dr. David Rotstein on: 4/19/10

Host Identification: NY 4236-10

**Common Name:** Humpback whale *Scientific Name:* *Megaptera novaengliae*

Age: 2-4 yrs Sex: male Size: 30 ft Weight: 13 tons

Host Location: Long Island, NY, USA

Parasite Location: left kidney

Initial Tissue Identification: parasite

Fixative: 70% etOH

Reason for exam: live animal stranding, euthanized, and field necropsy of code 2 animal


Case Notes: The case was well publicized in the media and on-line in the north-east region, which involved a young single humpback whale that stranded alive in the surf zone off the Hampton's on Long Island NY. The whale was suffering and no facilities for rehabilitation of such a large whale exist on the east coast. Towing the animal out to sea is dangerous, and only prolongs the animal's suffering. The whale was given medications for pain and distress and eventually euthanized.

tissue submitted to the NMLC becomes the property of the NMLC, may be archived, forwarded or destroyed in processing.


Processing: Three fragments of a nematode were provided for evaluation, the samples were cleaned in ethanol and imaged.

Tissue Inventory and Description: Three fragments of a nematode or nematodes are presented. Internal structures reveled ova with larva and the posterior portion of a female worm with a distal anus within a papilla and a vulvar opening 3.13mm from the anus. The cuticle is highly folded into crenulations with a clear outer cuticle, the worm is white to pale yellow.

Measurements:

width=2.03 - 2.49mm
space between crenulations of the cuticle 0.1-0.13 mm
anal papilla length = 0.65mm, width at base=0.89mm
distance from anus to vulva = 3.13mm
vulvar opening = 0.23mm (horizontal oval slit)
length of posterior bulb = 2.71mm, width at constriction = 1.11mm
ova: morulated to larvated n=1
30.64um x 25.77um; area 620.05 sq um; perimeter=88.64 um

Measurements were taken with a Diagnostic Instruments Inc. digital Insight Spot model 18.2 color mosaic 2 MP three chip camera, on a Nikon SMZ800 dissection microscope illuminated by a Nikon NI-150 fiber optic system, or AO compound microscope. Images are processed with SPOT software version 4.6 and may be post-processed with Adobe Photoshop Elements 6.0 on a MacBook Pro OSX (10.5.8).

Confidence Codes
A: proven, findings documented, complete taxon review, defendable and publishable
B: diagnostic, referenced morphological characteristics
C: primary rule out, conclusion based on limited data but can be logically assumed, common host-parasite relationship
D: best guess, significant data or documentation missing to support claims
E: insufficient data or documentation to prove this opinion but it is likely based on past experience or on assumption

This report was generated in part with diagnostic equipment acquired with a generous grant from Entergy and we gratefully acknowledge their support of marine science and the NMLC.

ID: (Taxonomic system) [confidence code]{identifying characteristics}

| | |
|---|---|
| Kingdom | Animalia (ITIS) [A] |
| Phylum | Nematoda (ITIS) [A] |
| Class | Secernentea (ITIS) [A] |
| Order | Spirurida (ITIS) |
| Family | Tetrameridae (ITIS) or Crassicaudidae(Skrjabin adn Andreeva, 1934) |
| Genus | *Crassicauda* |
| Species | species |

Host/Parasite Relationship: Parasites of *Megaptera novaengliae (humpback whale)*

**Acanthocephala:**

*Bolbosoma sp.*
*Bolbosoma balaenae*
*Bolbosoma turbinella*
*Corynosoma sp.*

**Cestoda:**

*Diplogonoporus balaenoptera*

**Nematoda:**

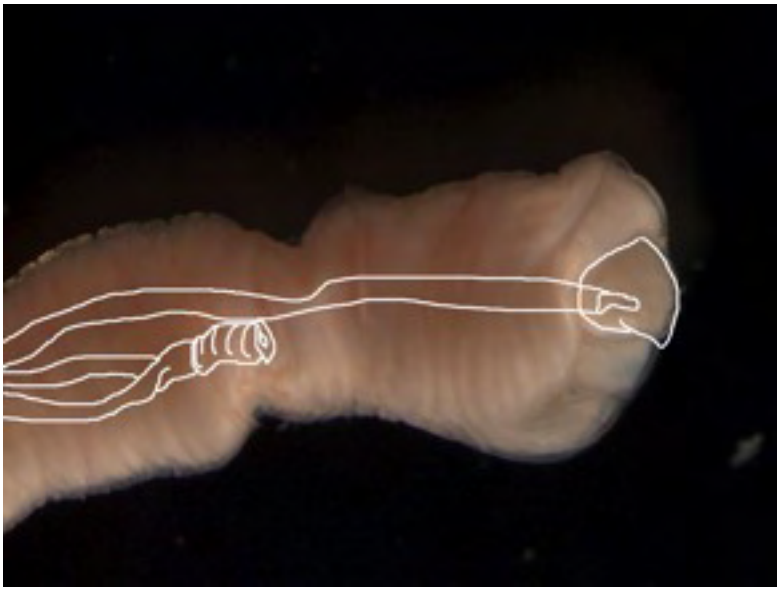
*Anisakis sp.*
*Crassicauda sp.*
*Crassicauda boopis*
*Crassicauda crassicauda*

**Trematoda:**

*Lecithodesmus sp.*

**Amphipoda:**

*Cyamus boopis*
*Cymus erraticus*



Host Notes: A history of the event or other test results was not provided and may be pending.

Th ere are only three species of nematodes described from this host. The samples examined are nematodes. Either the sample is one of the described species or is a new species altogether, which is quite possible as the parasites of whales are in need of further study, or one of the listed worms. This sample is not consistent with *Anisakis* in shape or location and can be ruled out. The parasite is consistent with *Crassicauda*, but this genus is in need of further revision and study.

Parasite Notes and similar species:

Th e genus *Crassicadua* has six species, three of which are incompletely described (*C. bennetti*, *C. grampicola*, and *C. magna*).

*C. crassicauda* found in the lower urinary tract, and in the bottlenose dolphin, smaller then *C. boopis*
*C. giliakiana* found in the kidneys of beluga and bottlenose whales
*C. bennetti* found in the kidney of the bottlenose whale
*C. grampicola* found in Risso's dolphin peri-otic sinuses, anal papilla with a conical tip
C. *boopis* (syn. *C. pacifica*) found in the kidney of large baleen whales
*C. magna* found in the pigmy sperm whale

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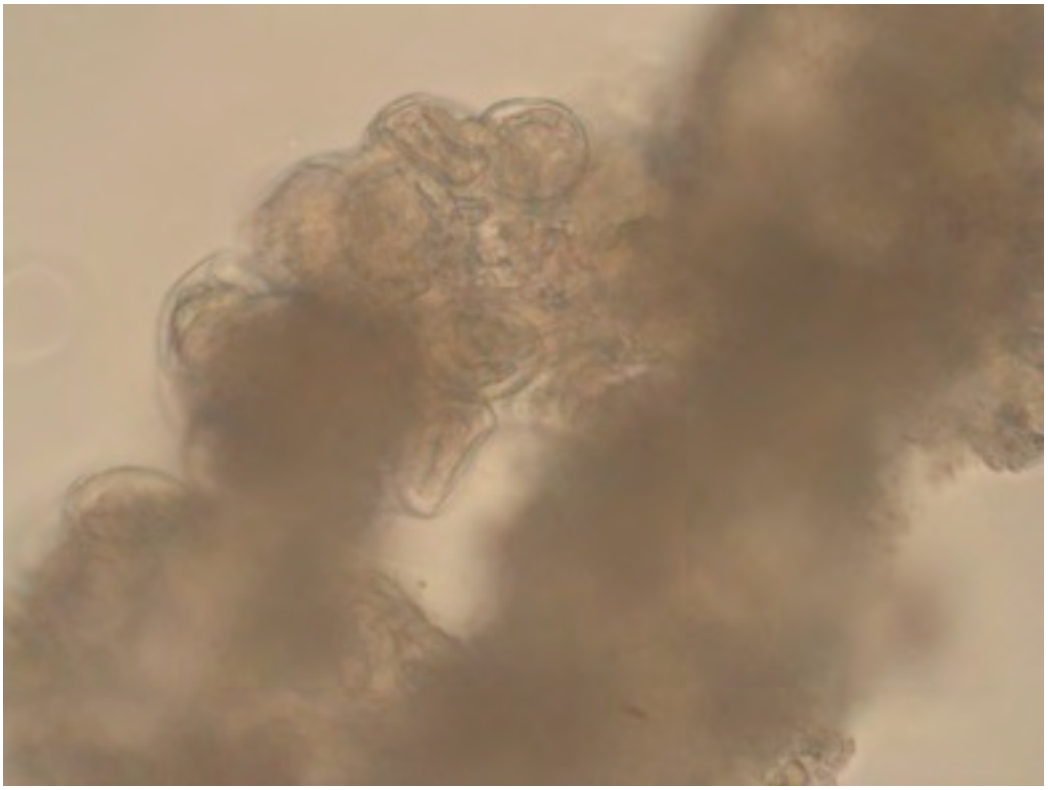
Based on the literature (Baylis 1922; Delyamure 1955)this sample would be *C. boopis* (host: humpback whale. location: kidney) but the observed specimen has characteristics more consistent with *C. grampicola* (anal papilla with conical tip, smaller egg), and is almost too small to be *C. boopis* (width insufficient, and distance from vulva to terminal tip too small). Larva were observed so at least one fragment was from an L5 but perhaps this nematode was an early L5 and had not grown into the full characteristics of a fully mature *C. boopis*.

Th is parasite is transversely striated. The genital constriction is not pronounced (1.11mm) and is located 2.71mm from the anus with a distance of 3.13mm from the anus to the vulva. For *Crassicauda boopis* (Lambertsen 1985) this distance is 6-7.5mm and no anal papilla is described. The ova are morulaed to larvated and are 30 x 25um which is smaller then those described for *C. boopis* at 55 x 37um.



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ID via: host/parasite relationship, common knowledge, taxon review
Natural History Significance: The tissue nematodes of cetaceans are a poorly understood and important issue for biology, medicine, and conservation of whales. The following is taken from the Humpback whale recovery plan.

**FINAL RECOVERY PLAN FOR THE HUMPBACK WHALE (*MEGAPTERA NOVAEANGLIAE*)**
**NOVEMBER 1991 PREPARED BY THE HUMPBACK WHALE RECOVERY TEAM**

**1.43** Implement base-line study of parasite load in whale tissues and contaminant levels in tissues of whales and prey.

**3.512** Identify and quantify causes of natural mortality in juvenile and adult hum~back whales. Information resulting from activities recommended in this Plan will lead to a better understanding of natural mortality. Episodic events,such as entrapment of humpback whales in ice along the coast ***of*** Newfoundland, should be documented when they occur. Better information on parasite load, biotoxin occurrence and effects and natural pathology of stranded whales may shed new light on the role of those factors in causing death.

Veterinary Significance: If infection is limited to a single kidney, even an extensive unilateral infection may not cause azotemia and renal insufficiency, but may still be a source of chronic inflammation and discomfort to the animal. By occupying the renal vasculature a systemic effect could also be felt, with the possible of chronic release of inflammatory mediators from the vessel lining, decreased perfusion, hypertension, and through the development of thrombi and emboli. Bilateral involvement is a serrious veterinary condition and sufficient to results in stranding and perhaps death of fin whales(Lambertsen 1986), where infection with *C. boopis* is common.
Treatment Recommendations: While treatment recommendations are not applicable to necropsy cases, treatment of the disease Carasscaudosis is problematic at best. Killing the worms may be possible with antihelminthic preparations such as fendbendazole, however, the host would still have to deal with the dead worms within the tissue, which might cause more inflammation following death then was present when the worm was alive, and in the case of renal parasites, obstruction as a result of treatment must be considered. Surgical removal of the worms is currently not imaginable as the worms can not be easily dissected at necropsy, any approach would be too destructive to organs. Also, ivermectin has been reported to cause serious toxicities in cetaceans and should not be used.
Veterinary Case Notes: pending review
Case Reporter: Sea Rogers Williams VMD
Disposition: archived / forwarded / histology / destroyed
preliminary report date: 4/28/10 fi nal report date: pending notification: x

This report was generated in part with diagnostic equipment acquired with a generous
grant from Entergy and we gratefully acknowledge their support of marine science and the NMLC.

# Bibliography

Baylis, H. A. (1922). Note on the Habitat and Structure of *Crassicauda* [Nematoda]. Parasitology. **14:** 9-12.

Delyamure, S. L. (1955). Helminthofauna of Marine Mammals (Ecology and Phylogeny). Moskva, Izdatel'stvo Akademii Nauk SSSR.

Lambertsen, R. H. (1985). "Taxonomy and Distribution of a Crassicauda Species (Nematoda: Spirurida) Infecting the Kidney of the Common Fin Whale (*Balaenoptera physalus* Linne, 1758)." Journal of Parasitology **71**(4): 485-488.

Lambertsen, R. H. (1986). "Disease of the Common Fin Whale (*Balaenoptera physalus*): Crassicaudiosis of the Urinary System." Journal of Mammalogy **67**(2): 353-366.

This report was generated in part with diagnostic equipment acquired with a generous grant from Entergy and we gratefully acknowledge their support of marine science and the NMLC.

Entergy

**Patient Info:**

**Name:** Mnovangliae
**Chart No:**
**Owner:** Ny4236 10
**Doctor:**

**Species:** Mammal
**Breed:**
**Age:**
**Sex:** U

**Hospital:**

Riverhead Found-Marine Res & Pres
467 E Main St
Riverhead, NY 11901
**Antech ID:** 7798

**Lab:**

Antech Diagnostics
1111 Marcus Avenue
Lake Success, NY 11042
**Reported:** 04/13/10 01:49 PM
**Received:** 04/10/10

| Accession No. | Doctor | Owner | Pet Name |
|---|---|---|---|
| **NYAA85516313** | | **Ny4236 10** | **Mnovangliae** |

| Test | Results | Adult Reference Range | L | Normal | H |
|---|---|---|---|---|---|
| **Corneal Cultures** | **#1** | **#2** | **#3** | **#4** | **#5** |
| Bacitracin | S | | | | |
| Chloramphenicol | S | | | | |
| Ciprofloxacin | S | | | | |
| CEFAZOLIN | S | | | | |
| Gentamicin | S | | | | |
| Norfloxacin | S | | | | |
| Ofloxacin | S | | | | |
| Polymyxin B | S | | | | |
| Tobramycin | S | | | | |
| Neomycin | S | | | | |
| Tetracycline | S | | | | |
| **Aerobic Culture & MIC** | **#1** | **#2** | **#3** | **#4** | **#5** |
| Source: | Eye | | | | |
| Preliminary #1 | 04/11/2010 | | | | |
| Organism #1 | | | | | |
| Coagulase negative Staphylococcus spp.<br>Light growth | | | | | |
| Final Report | 04/13/2010 | | | | |

**Patient Info:**

**Name:** Mnovangliae
**Chart No:**
**Owner:** Ny4236 10
**Doctor:**

**Species:** Mammal
**Breed:**
**Age:**
**Sex:** U

**Hospital:**

Riverhead Found-Marine Res & Pres
467 E Main St
Riverhead, NY 11901
**Antech ID:** 7798

**Lab:**

Antech Diagnostics
1111 Marcus Avenue
Lake Success, NY 11042
**Reported:** 04/14/10 07:32 AM
**Received:** 04/10/10

| Accession No. | Doctor | Owner | Pet Name |
|---|---|---|---|
| **NYAA85567484** | | **Ny4236 10** | **Mnovangliae** |

| Test | Results | Adult Reference Range | L | Normal | H |
|---|---|---|---|---|---|
| **Sensitivities** | **#1** | **#2** | **#3** | **#4** | **#5** |
| Amikacin | S | | | | |
| Ampicillin | R | | | | |
| Clavamox | S | | | | |
| Chloramphenicol | S | | | | |
| Carbenicillin | S | | | | |
| Cephalothin | R | | | | |
| Doxycycline | S | | | | |
| Enrofloxacin | S | | | | |
| Gentamicin | S | | | | |
| Neomycin | S | | | | |
| Trimethoprim/Sulfa | S | | | | |
| Tetracycline | S | | | | |
| Marbofloxacin | S | | | | |
| **Aerobic Culture & MIC** | **#1** | **#2** | **#3** | **#4** | **#5** |
| Source: | Blowhole | | | | |
| Preliminary #1 | 04/11/2010 | | | | |
| No growth after 24 hours. | | | | | |
| Preliminary #2 | 04/12/2010 | | | | |
| Organism #1 | | | | | |
| Listonella anguillarum<br>Light growth | | | | | |
| Final Report | 04/14/2010 | | | | |

# ATHENS DIAGNOSTIC LABORATORY
# COLLEGE OF VETERINARY MEDICINE
## UNIVERSITY OF GEORGIA
## ATHENS, GA 30602

**Phone #: 706-542-5568** **Fax #: 706-542-5977**

**Owner:** RIVERHEAD FOUNDATION ( RFMRP)
467 EAST MAIN STREET
RIVERHEAD, NY 11901

**Veterinarian:** DR. ROBERT PISCIOTTA
RIVERHEAD FOUNDATION (RFMRP)
467 EAST MAIN STREET
RIVERHEAD, NY 11901

**Accession Number:** A11-00641
**Reference Number:**
**Received:** 07/07/2010 **Finalized:** 07/09/2010
**Species:** EXOTIC - WHALE, DOLPHIN,
**Breed:** WHALE
**Sex:** MALE **Age:** JUVENI
**Animal ID:** NY4236-2010
**Case Ref #:**
**Specimen:** SEE SPECIMEN ID

## MOLECULAR BIOLOGY RESULTS

**Morbillivirus PCR**

**COMMENTS** A negative result indicates that morbillivirus RNA was not detected in the sample tested. Because of possible uneven distribution of virus in many tissues, a negative result on a single sample does not completely rule out morbillivirus infection in the animal.

**Morbillivirus PCR**

| ANIMAL ID | SPECIMEN DESC | PCR RESULT |
|---|---|---|
| Ny4236-2010 | Fresh Tissue | NEGATIVE -TBLN |
| Ny4236-2010 | Fresh Tissue | NEGATIVE -VITREOUS OS |
| Ny4236-2010 | Fresh Tissue | NEGATIVE -CSF |
| Ny4236-2010 | Lung | NEGATIVE |
| Ny4236-2010 | Spleen | NEGATIVE |

Reported By: Ingrid Fernandez

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**Species:** EXOTIC - WHALE, DOLPHIN,
**Breed:** WHALE
**Sex:** MALE **Age:** JUVENI
**Animal ID:** NY4236-2010
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**Specimen:** SEE SPECIMEN ID

## MOLECULAR BIOLOGY RESULTS

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|---|---|---|
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| Ny4236-2010 | Fresh Tissue | NEGATIVE -VITREOUS OS |
| Ny4236-2010 | Fresh Tissue | NEGATIVE -CSF |
| Ny4236-2010 | Lung | NEGATIVE |
| Ny4236-2010 | Spleen | NEGATIVE |

Reported By: Ingrid Fernandez

**NY Humpback Whale Stranding**
**Algal Toxins Report**

**Date:** 18 August 2010

**Documented by:** Spencer Fire
NOAA National Ocean Service, Marine Biotoxins Program
219 Fort Johnson Road, Charleston, SC 29412
(843) 762-8574 <spencer.fire@noaa.gov>

## A. Suspected Event and Sample Details

Samples from a humpback whale (*Megaptera novaeangliae*) stranding in East Hampton, NY on 6 April 2010 were sent to NOS Charleston by Kimberly Durham of the Riverhead Foundation for Marine Research and Preservation. Sample types analyzed included urine, bile, stomach contents and intestinal contents. Samples were assigned NOS ID numbers (NYM01-05) upon receipt in preparation for toxin extraction and analysis. A complete sample list is given in the data summary below (section H).

## B. Findings

**No biotoxins were detected in any of these samples.**

## C. Saxitoxin (STX) Analyses

**1a. Preparation of Urine Sample:** The urine sample was centrifuged at 10000 x *g* and filtered (0.2 µm) prior to analysis. The sample was prepared for analysis by Spencer Fire.

**1b. Preparation of Bile, Stomach Contents and Intestinal Contents Samples:** Samples were extracted by combining and homogenizing at a minimum a given weight of sample (typically 5-10 grams) with an equal volume of 0.1N hydrochloric acid. Homogenized samples were boiled for five minutes then volumetrically resuspended (typically 15-25 mL). The pH of the cooled mixture was measured and adjusted if necessary to fall between 3.0 and 4.0. These extracts were centrifuged at 3400 x *g*, then the supernatant was collected and syringe filtered (0.45 µm). Samples were prepared for analysis by Spencer Fire.

**2. RBA Methods:** The acidic aqueous extracts and filtered samples were analyzed in a STX receptor binding assay (RBA). The receptor binding assay measures competition between radiolabeled STX and sample or FDA standard (S. Hall, USFDA/CFSAN, Washington, DC) for binding to the voltage-gated sodium channel, the pharmacological target of STX, to determine the total saxitoxin-like activity of the sample. Receptor binding analysis was performed by Trey Knott.

**3. RBA Results:** Saxitoxin-like activity was not identified in any of the samples analyzed. The detection limit of this method was 34 ng STX equivalents per gram of extracted sample or 22 ng STX equivalents per milliliter of urine.

## D. Domoic Acid (DA) Analyses

**1a. Preparation of Urine Sample:** The urine sample was centrifuged at 10000 x *g* and filtered (0.2 µm) prior to analysis. Sample clean-up was performed by solid-phase extraction (SPE) using Varian 50 mg C18 columns. The sample was prepared for analysis by Bennie Haynes.

**1b. Preparation of Bile, Stomach Contents and Intestinal Contents Samples:** Samples were extracted by adding four volumes of 50% aqueous methanol to the sample volume, homogenizing, and then probe sonicating on ice for 2 min. These extracts were then centrifuged at 3400 x *g*, and the supernatant collected and syringe filtered (0.45µm) prior to analysis. Sample clean-up was performed by solid-phase extraction (SPE) using Varian 200 mg C18 columns. Samples were prepared for analysis by Bennie Haynes.

**2. LC-MS Methods:** The methanolic extracts and filtered samples were analyzed for the presence of domoic acid using tandem mass spectrometry coupled with liquid chromatographic separation (LC-MS/MS). This method utilized reversed phase chromatography, using an Agilent 1100 HPLC coupled to an ABI-SCIEX API-4000 triple quadrupole mass spectrometer in ESI+ mode. Chromatographic separation was performed on a Phenomenex Luna C18(2), 5µ, 150mm x 2 mm column. Mobile phase consisted of water and acetonitrile in a binary system, with 0.1% formic acid as an additive. The elution gradient was as follows: 3 min of 5% acetonitrile, with a linear gradient to 40% acetonitrile at 16 min, 95% acetonitrile at 18 min, held for 5 min, then returned to initial conditions at 24min

and held for 5 min before the next injection. In order to reduce mass spectrometer contamination, a diverter valve was used to switch the LC eluant to the waste container except for the 6 minute window of LC eluant bracketing the retention time for DA that was sent to the MS. Retention time of DA in samples was determined based on the retention time observed with a certified DA reference standard from the Institute for Marine Biosciences, NRC Canada (Halifax, NS). The detection of domoic acid by MS was achieved by Multiple Reaction Monitoring (MRM) method with Turboionspray interface. Four MRM transitions from protonated domoic acid were monitored: *m/z* 312 → 266, *m/z* 312 → 248, *m/z* 312 → 193, and *m/z* 312 → 161. LC/MS analyses were performed by Zhihong Wang.

**3. LC-MS Results:** DA was not identified in any of the samples analyzed. The limit of quantification (LOQ) of this method was 0.2 ng domoic acid per mL urine or 2.5 ng per gram sample, with a signal to noise ratio slightly above ten for standards.

**E. Brevetoxin (PbTx) Analyses**

**1a. Preparation of Urine Sample:** The urine sample was centrifuged at 10000 x *g* and filtered (0.2 µm) prior to analysis. The sample was prepared for analysis by Spencer Fire.

**1b. Preparation of Stomach Contents and Intestinal Contents Samples:** Samples were homogenized and extracted twice in 3 volumes of acetone, filtered via a 0.45 µm Acrodisc syringe filter, evaporated, resuspended in 80% aqueous methanol (6 or 30 mL), twice solvent partitioned with 30 mL hexane, and the methanolic fraction collected, evaporated and resuspended in 5 mL of 100% methanol. Extracts were stored at -20$^{o}$C until analysis. Extractions were performed by Spencer Fire.

**2. ELISA Methods:** Filtered urine and extracted samples were analyzed using a direct competitive enzyme-linked immunosorbent assay (ELISA) for PbTx. The ELISA utilizes cross-reactivity of PbTx to anti-PbTx antibodies to determine PbTx-like activity in a sample. Quantitation is determined via competition between PbTx in the sample and PbTx conjugated to a signal enzyme for binding to anti-PbTx antibodies. Analyses were conducted by Spencer Fire.

**3. ELISA Results:** PbTx-like activity was initially detected in one sample (NYM05, intestinal contents). However, negative control material from humpback whales was unavailable to run in parallel to determine possible matrix effects in the assay. The sample was subsequently sent for confirmation of toxin by LCMS methods. The limit of detection for this assay was 1 ng PbTx per gram of sample or mL of urine.

**4. LC-MS Methods:** Selected samples cleaned by C18 solid phase extraction cartridges (Varian) were analyzed by liquid chromatography/mass spectrometry (LC-MS) for parent PbTx toxins and metabolites. Liquid chromatography separations were performed on a Luna C8(2) 150 × 2 mm column using an Agilent Technologies Model 1100 LC system. Mobile phase consisted of water (A) and acetonitrile (B), with 0.1% formic acid or formic acid additive with gradient elution. The mobile phase flow rate was 0.2 mL/min. The eluant from LC was analyzed by an Applied Biosystems/MDS Sciex 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer equipped with a TurboV$^{TM}$ interface. The analysis of PbTx congeners and metabolites by mass spectrometry was achieved by multiple reaction monitoring (MRM).Brevetoxin congeners monitored included PbTx-1, -2, -3, -7, -9, oxidized PbTx-2, open A-ring PbTx-1, -2, -3, -7, -9, open A-ring oxidized PbTx-2, cysteine-PbTx-B (A) and its sulfoxide, glutathione conjugate (B type). LC/MS analyses were performed by Zhihong Wang.

**5. LC-MS Results:** Brevetoxin congeners were not detected in any of the samples analyzed. The limit of quantification (LOQ) of this method was approximately 0.5 ng brevetoxin per mL urine or per gram sample, with a signal to noise ratio slightly above ten for standards.

**F. Okadaic Acid (OA) Analyses**

**1a. Preparation of Urine Sample:** The urine sample was centrifuged at 10000 x *g* and filtered (0.2 µm) prior to analysis. The sample was prepared for analysis by Spencer Fire.

**1b. Preparation of Stomach Contents and Intestinal Contents Samples:** Samples of stomach contents and intestinal contents (1-2g of material) were homogenized and extracted in 3 volumes of 100% methanol, followed by centrifugation at 3000 x *g* for 5 min. The methanolic supernatants were filtered with a 0.2 µm syringe filter in preparation for analysis. Samples were prepared by Spencer Fire.

**2. LC-MS Methods:** Samples were analyzed for the presence of okadaic acid (OA) and its two congeners (DTX1 and 2) using liquid chromatography (Agilent 110 series HPLC, Palo Alto, CA) coupled with tandem mass spectrometry (Applied Biosystems/MDS Sciex, Foster City, CA). For quantitation of okadaic congeners, LC separation was performed on hypersil C8 BDS, 3µm, 50 × 2.1 mm column, (Thermo Electro, Waltham, MA) using a mobile phase of water (A) and acetonitrile/water (95:5, V/V) (B), with 2 mM ammonium acetate and 50 mM formic acid as an additive under gradient elution at a flow rate of 0.2 ml/min (linear gradient from 30% B at 0 min to 90% B at 8 min, hold for 3 min, then return to 30% B at 12 min and hold for 5 min), MS detection was in negative ion multiple reaction monitoring (MRM) mode using an API 4000 triple quadrupole mass spectrometer (for OA and DTX-2 with MRM transitions of *m/z* 803.5 → 151.0 and 255.1, for DTX-1 with MRM transitions of *m/z* 817.5 → 151.0 and 255.1) with limit of quantitation better than 1ng/ml with 5 µl injection. For further confirmation, dried methanolic extracts with C18 solid phase extraction cleanup (Varian, 500 mg, 3 ml) were injected to a Luna C8(2) column (150 × 2 mm, 5 µm, Phenomenex, Torrance, CA, USA) using a mobile phase of water (A) and acetonitrile (B), with 0.1% acetic acid as an additive for gradient separation ( 2 min of 45% B, linear gradient to 80% B at 30 min, 95% B at 31 min, hold for 5 min, then return to 45% B at 37 min and hold for 8 min), MS detection was in negative ion enhanced product ion mode with the function of linear ion trap using a 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer. LC/MS analyses were performed by Zhihong Wang.

**3. LC-MS Results:** Okadaic acid congeners were not detected in any of the samples analyzed. The limit of quantification (LOQ) of this method was approximately 1 ng toxin per mL urine or per gram sample, with a signal to noise ratio slightly above ten for standards.

## H. Data Summary

**Table 1.** Biotoxin concentrations (ng/g or ng/mL) detected by LCMS, ELISA and RBA methods. <dl = below detection limit. pos. = positive but unconfirmed by LCMS.

| NOS ID | Animal ID | Stranding location | Species name | Common name | Date | Sample type | OA-LCMS | PbTx ELISA | PbTx-LCMS | STX RBA | DA-LCMS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NYM01 | NY4236-2010 | NY - East Hampton | *Megaptera novaeangliae* | Humpback whale | 4/6/10 | bile | <dl | | | <dl | <dl |
| NYM02 | | | | | | urine | <dl | <dl | | <dl | <dl |
| NYM03 | | | | | | stomach contents | <dl | <dl | | <dl | <dl |
| NYM04 | | | | | | intestinal contents | <dl | | | <dl | <dl |
| NYM05 | | | | | | intestinal contents | <dl | pos. | <dl | <dl | <dl |

**Figure 1**. Stranding location.